MICROBIAL UTILIZATION OF CATHODIC HYDROGEN AND RELATED CORROSION

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To the memory of my father, Pedro Rafael Sifontes Lopez.

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Microbial corrosion is the deterioration of a material by corrosion processes that occur directly or indirectly as a result of the activity of microorganisms. Although it is a widely recognized phenomenon in many industrial processes worldwide, the fundamental corrosion mechanisms are not understood, it is not well defined, and the microbial effect has not been quantified. The objective of this research was to show a relationship between cathodic hydrogen utilization by bacteria and corrosion at its onset for the purpose of improving its understanding and attempting to quantify the biological component of corrosion.

Two new experimental flowthrough and batch bioreactor systems were evaluated to reproduce anaerobic microbial corrosion. Both reactors were able to reproduce microbial corrosion in 24 hr. However, the batch system offered the

better alternative to study microbial corrosion at an attractive economic operation. Since microbial corrosion is difficult to separate from pure electrochemical corrosion and electrochemical measurements offers a nondestructive technique to measure corrosion, the batch bioreactor was instrumented for electrochemical measurements and for physicochemical analysis.

Results from potential-time curves, dissolved iron profiles, and differential corrosion currents indicated that metals were reactive to the presence of bacteria. However, the results were not adequate to quantify the biological component of corrosion. Significant increases in corrosion rates, observed during the addition of a terminal electron acceptor, suggested a catalytic effect on the bacterial hydrogenase system. The observation of cracks on steel samples suggested the occurrence of hydrogen embrittlement.

The results of this research are important because they offer a new approach for the investigation of microbial corrosion, the triad cell offers an opportunity to quantify the biological component of corrosion, and a new mechanism was proposed for the understanding of the onset of anaerobic microbial corrosion.

CHAPTER 1
INTRODUCTION

Objectives

The overall objective of this research was to show a direct relationship between corrosion of metals and hydrogen utilization by bacteria during the onset of microbial corrosion, for the purpose of improving our understanding of the phenomenon. A new approach to the investigation of microbial corrosion was selected to break ground in an alternative direction to the one established over the past century and for a better comprehension of the fundamental microbial corrosion mechanisms.

The specific objectives of the study were to

1) evaluate new experimental flowthrough and batch bioreactors to adequately reproduce anaerobic microbial corrosion,

2) quantify the biological component of corrosion at its onset by measuring indirectly hydrogen uptake by bacteria and correlating it to total metal corrosion, using nondisrupting electrochemical techniques,

3) incorporate into a comprehensive microbial corrosion model all concepts developed during the investigation.

Justification

The ability of microorganisms to induce or influence the deterioration of metals has been known for over a century, and now it is a widely recognized problem in most industrial processes worldwide. The involvement of microorganisms in metal deterioration has led to the question of how biological agents affect the classical corrosion mechanisms.

The true economic impact of metals deterioration is very difficult to determine. However, some estimates have been made in the USA and the UK. In the USA, the cost of corrosion estimated by Poff (1985) and a projection by the National Bureau of Standards for the year 1985 was approximately $140 billion and $170 billion, respectively. As for microbial metal deterioration, studies performed by Butlin et al. (1952), Allred et al. (1959), Booth (1964), and the National Corrosion Service (Wakerly, 1969) estimated that metallic corrosion caused by microbial intervention in a wide range of industrial cases in the UK was on the order of 70%, 77%, 50%, and 10%, respectively, of the total corrosion problem. In the USA, Paternaude (1985) indicated that 50% of the steel culvert pipe corrosion in Wisconsin was due to sulfate reducing bacteria. If the lowest 10% is selected, the cost of microbial corrosion represents a $17 billion per year problem in the USA alone. Although such calculations may be open to objections on matters of detail, the magnitude is likely to

stay high whatever route is followed to arrive at a final cost because the above costs include replacement, prevention, and maintenance but exclude losses of time, money, natural resources, human suffering, and death due to equipment failure.

Metallic corrosion is a natural process in which metals return to their natural, oxidized states. It is an interdisciplinary field of engineering and science where a knowledge of the metallurgy of the metal, the environmental conditions, the chemical composition, and the electrochemistry of the system, are essential to understanding the process. For example, hydrogen embrittlement is too complex for one of the above disciplines to explain, and despite several decades of investigation it is still unclear why hydrogen embrittles some metals and alloys and not others (Oriani, 1987; Wilde and Kim, 1986). Most corrosion processes are essentially surface electrochemical mechanisms common to most metals in aqueous, or at least humid, environments. This conclusion was first reached by Whitney (1903). During the manufacturing process of metals from their ores, metals are converted to a reduced state, which makes them thermodynamically unstable in the presence of oxygen, except gold.

Microbial corrosion is defined as the deterioration of materials by natural processes that are directly or indirectly related to the activity of microorganisms. The microbial corrosion phenomenon has been known for nearly a century. It

has been reported to exist almost anyplace that microorganisms colonize. In the process industry, it can happen inside and outside of equipment and in aerobic or anaerobic environments. Microbial corrosion is a diverse and complex phenomenon, and the literature associated with it tends to be just as diverse and complex. Microbial corrosion was well recognized and established as a serious problem in the 1970s (Tatnall, 1981). Microbial corrosion is difficult to separate from pure electrochemical corrosion. Microorganisms involved in microbial corrosion generally do not lead to a new form of corrosion, but to a stimulation of the normal electrochemical corrosion process. Consequently, if the microbes interfere with an electrode process, with their own metabolism or presence, it is a direct effect, otherwise it is an indirect effect.

To demonstrate microbial corrosion, the presence of microorganisms must be shown to induce or influence metal loss during corrosion. Unfortunately, microbial corrosion in real cases is very difficult to reproduce, extremely complex, and difficult to model (Tatnall, 1988). Laboratory-based controlled experiments using defined media and characterized pure cultures do not often yield the expected results because a broad range of variables makes it difficult to simulate the dynamic natural environment (Allsopp and Seal, 1986).

The study of microbial corrosion has had a tendency to consider processes in isolation rather than actual microbial

corrosion cases. For instance, there are many studies dealing with the effect of a single species of microorganisms on metal corrosion (Tatnall, 1988). While such investigations are valuable in elucidating mechanisms, they give little insight into the wider biological/metal/fluid interactions. Although recent reappraisal of the role of bacterial consortia together with studies of the effect of such consortia have improved our knowledge of the microbial corrosion process, few studies of microbial corrosion look beyond bacterial consortia and surface corrosion effects to wider biological/metal/fluid interactions. Furthermore, any living or indeed dead organism that becomes associated with a metal surface immersed in an electrolyte has the potential to influence the corrosion of that metal. Edyvean (1988) in his recent work discusses the interaction of both bacteria and macro- and micro-algae in the fouling community on steel substrata in sea water.

Most engineering materials in general use are susceptible to some form of microbial corrosion. Several of the metals and alloys reported as being susceptible to microbial corrosion include iron, copper, aluminum, nickel, cobalt, and zinc and their alloys (Gabrielli, 1988; Zamanzadeh et al., 1989; Griffin et al., 1989). The industries affected by microbial corrosion have been identified as wastewater facilities, water flood control systems, petrochemical equipment, cooling water systems, underground structures and pipelines, aircraft fuel systems, ships and marine structures,

chemical process industries, power generation industries, and paper mills.

From case histories of microbial corrosion, the forms of corrosion that are stimulated by the interaction of microorganisms with metals range from general pitting corrosion, crevice corrosion, and stress corrosion cracking to enhancement of corrosion fatigue, intergranular stress cracking, and hydrogen embrittlement with cracking (Sifontes and Block, 1991).

The fundamental mechanisms that drive microbial corrosion are not properly understood nor have they been well defined. Consequently, additional research to understand them is required. Most work to date in microbial corrosion has dealt with reports of case histories or observations incidental to the main study, with poor documentation regarding the physical, chemical, and microbiological conditions under which it occurred (Tatnall, 1981, 1988).

Data from the literature surveyed suggest that qualitative relationships exist between the surface properties of the various metals and their alloys and the extent of biological response in relation to corrosion. The fact is that it is not well known why some metals are more susceptible to microbial corrosion than are others and why the microbial corrosion effect of a particular microorganism is different from the microbial corrosion effect of similar or different species.

Background

The literature indicates that the first time a mechanism for microbial corrosion was proposed was in 1934 by von Wolzogen Kuhr and van der Vlught. This theory initiated systematic studies on microbial corrosion and identified formally the components of the microbial corrosion phenomenon, namely, the metal surface, the suspended fluid, and the microorganisms. They proposed that sulfur reducing bacteria accelerated the corrosion of ferrous metals by cathodic depolarization, that is, by removing adsorbed hydrogen from the cathodic surfaces of the metal.

The theory appeared to be relatively simple to confirm by conventional electrochemical techniques. However, qualitative confirmation has been obtained only for specific hydrogenase-positive species of microorganisms such as sulfate reducing bacteria (Booth and Tiller, 1960) and methanogens (Daniels et al., 1987). Belay and Daniels (1990) reported that besides iron, other metals (Al, Zn, Ni, and As) can also be oxidized via cathodic depolarization.

In the work performed by Daniels and coworkers (1987), it was demonstrated that methanogenic bacteria use either pure elemental iron or iron from mild steel as a source of electrons in the reduction of carbon dioxide to methane. These bacteria use the oxidation of iron for energy generation and growth. The mechanism of iron oxidation is cathodic

depolarization, in which electrons from iron and hydrogen ions from water produce molecular hydrogen, which is then released for use by the methanogens.

The extent of bacterial adhesion is determined by the surface properties of the phases involved and the need of bacteria to locate energy sources. Bacterial cells will stick to most surfaces, whether the surfaces are those of other cells or merely inert material such as metals. In nutrient-poor environments (e.g., water transmission pipelines), most bacteria grow attached to surfaces mainly due to hydrophobicity.

It is the belief of some researchers that initially, sessile bacteria adhere randomly to metal surfaces by means of their production of extracellular polysaccharides. The continued production of the polysaccharide and the reproduction of the bacteria lead to the development of biofilms in which a consortium of cells interact in a hydrated matrix of anionic polysaccharide polymers that provide protection from natural or synthetically produced antimicrobial agents (Costerton and Geesey, 1985).

As a bacterium begins to proliferate within biofilms, its metabolic products stimulate the growth of other organisms. As the different microorganisms develop, molecular or proton exchanges occur; consortia such as these have been detected and associated with microbial corrosion of metallic surfaces.

In many neutral solutions the corrosion of the common structural metals appears to be associated with the flow of electric currents between various parts of the metal surface at finite distances from one another (Mears and Brown, 1941). This statement is supported by much evidence in the case of steels, where the quantities of current flowing during corrosion account for the amount of corrosion that occurs. In other words, the corrosion of metals and their alloys in neutral solutions is electrochemical in nature. Furthermore, for corrosion to occur, it requires all four components of an electrochemical cell which include: 1) electrodes, 2) electrolyte, 3) potential difference, and 4) electrical continuity. When one of these conditions is regulated, then the corrosion process is controlled.

Among some of the nonbiological factors of microbial corrosion that corrosion engineers and scientists have associated with corrosion and have known for several decades are 1) impurities in the corroding metal, 2) grain boundaries, 3) orientation of grains, 4) differential grain size, 5) differential thermal treatment, 6) surface roughness, 7) local scratches and abrasions, 8) difference in shape, 9) differential strain, 10) differential pre-exposure to air or oxygen, 11) differential concentration or composition of the corroding solution, 12) differential aeration, 13) differential heating, 14) differential illumination, 15) differential agitation, 16) contact with dissimilar metals,

17) externally applied potential, and 18) complex cells (Mears and Brown, 1941). Those factors are closely related with the so-colled "eight forms" of corrosion, which include uniform, galvanic, crevice, pitting, intergranular, dealloying, erosion, and stress corrosion (Fontana and Green, 1987).

In addition to the above, there is always a lack of homogeneity at the metal-electrolyte interface during the microbial corrosion of metals. Consequently, several of the following specific interactions need to be recognized (Sato, 1987).

The Defective and Nonuniform Nature of Metallic Surfaces

During the formation of a metal or an alloy, different elements compose the molten metal. When solidification starts, metal is formed of crystalline grains that grow in size and they meet each other to form grain boundaries until the metal is all solid. Once the grains are formed, the overall energy of the individual metal atoms
lowers and the grain boundaries remain as sites of higher energy or higher thermodynamic instability (Smith, 1986).

The process of electron transfer is suspected to take place at metallic surface locations of higher thermodynamic instability such as the grain boundaries. This fact may be explained by the nature of the metallic bond where the metal ions occupy positions in the crystal lattice of the grains and the outer valence electrons are shared by the surrounding

metal atoms. These electrons are like a cloud that moves freely throughout the lattice and binds the crystal together. Consequently, microorganisms can take advantage of these electrons as their energy source for growth and affect the stability of the metal atoms.

In addition, the steel-making process and treatment influences the microstructure of the alloy by producing microcompositional differences that have different electrochemical behavior, which leads to the formation of localized corrosion. Other changes in the alloy's internal structure, such as aging and welding, may occur after it has been established during fabrication.

Among other metallurgical factors to be considered at the onset of microbial corrosion are the following

a) Composition of alloying elements and impurities (inclusions), cooling conditions and post heat treatments have a marked influence on their size, shape, number, and distribution. These constituents are never uniformly distributed throughout the alloy and certain elements tend to congregate in localized concentrations as second phase particles in grain boundaries, as pure compounds or as intermetallic compounds (Godard,1980). These particles play a major role in the corrosion behavior of alloys, especially in the case of pitting corrosion.

b) Surface contamination by mill scale during the rolling of the steel causes severe localized corrosion at breaks and

imperfections in the surface and may need to be removed before service. Steel can also be contaminated during forging with particles of metal from the forming equipment and consequently produce serious localized corrosion, too.

The Molecular Fluctuations of the Electrical Double Layer

For iron, there exists a spatial fluctuation of the electrical double layer potential on the metal surface in the order of 0.8 V for every 2-3 atomic distances (Sato, 1987). One possible reason is that, in addition to the heterogeneous metal surface, water dipoles tend to point nearly perpendicular to the metal surface, while the two nearest dipoles tend to align antiparallel to each other in order to minimize their dipole interaction energy. Consequently, reorientation of water dipoles in the electrical double layer occur in a time scale much greater than the time scale relevant to the electron fluctuation. In addition, water dipoles require greater time to reorient at surface defects sites where the adsorption energy of water dipoles is greater (Sato, 1987). This fact causes the electron transfer to take place unevenly on the metal surface. At certain surface defect sites, local enhancement of the electrical field in the electrical double layer may be maintained for a sufficiently long time so that the electrode reaction takes place preferentially at these sites, making them active reaction sites. Other sources of the spatial non-uniform electrical

double layer potential are specific ion adsorption. In this case, the ions divest of their hydration water molecules and come into direct contact with the metal surface. This causes a local enhancement (approximately 3 times) of the electrical field in the inner part of the electrical double layer. This phenomenon takes place preferentially at certain lattice or defect sites and will generate active spots for some electrode reactions.

The Mass Transport Perturbation in the Diffusion Layer

This dynamic perturbation causes the local ion concentration and the local electric field to fluctuate in the diffusion layer. In addition, when the corroding metal surface is usually covered with a porous corrosion precipitate film of hydrated metal oxides or insoluble salt, selective mass transport occurs, and this film either accelerates or decelerates further corrosion of the underlying metal. When those interactions are favorable to corrosion, chemical and/or electrochemical active surface sites occur that result in a corrosion activity. The nonuniform nature of the metal-electrolyte interface, whether passive or not, implies that the corrosion process takes place preferentially at specific sites that somehow differ energetically from other parts of the metal surface. These specific sites may be permanently localized or spatially fluctuate during the progress of corrosion.

As a result of the above, the microbial corrosion phenomenon is presumably initiated by the interaction of the metal, the bacteria, and the electrolyte at the onset of the process, followed by the formation of either differential concentration cells or local electrochemical corrosion cells within the biofilm, which further complicates the corrosion mechanism.

Microorganisms selected for this study had to contain a hydrogenase enzyme. Such microbes belong to the facultative lithotrophs, sulfur oxidizing bacteria, phototrophics, methanogens, and denitrifying bacteria. These bacteria are all hydrogen oxidizing bacteria that use molecular hydrogen as their electron donor. In anaerobic environments hydrogen is generated from the processes of fermentation or metal oxidation. Hydrogen oxidizing bacteria may grow autotrophically on hydrogen carbon dioxide as an electron acceptor.

Statement of the Problem

The cathodic depolarization theory of von Wolzegen Kuhr and van der Vlugt (1934) has become the center of attention of researchers in the field because it proposes a logical separation of the components of corrosion: biological, metallic, and fluid. As a result, much of the literature on

microbial corrosion has been influenced by this theory and has referred to it, either to criticize or prove it.

In work performed by Daniels and coworkers (1987), it was demonstrated that methanogenic bacteria used hydrogen produced from iron oxidation of carbon dioxide to methane. The mechanism of iron oxidation is cathodic depolarization, in which electrons from iron, and hydrogen ions from water produce molecular hydrogen that is then released and used by methanogens.

Most important, according to the literature reviewed, nobody has been able to determine how microorganisms induce or influence the corrosion process. Inspired by the cathodic depolarization theory and by Daniels and coworkers' demonstration, this research indicated that it might be feasible to measure and correlate total metal corrosion and hydrogen uptake by bacteria.

The project was initiated while evaluating some new bioreactor systems. In an attempt to understand and quantify the biological component of corrosion, an experimental system based on the batch bioreactor, which includes electrochemical measurement devices to study the utilization of cathodic hydrogen by bacteria and its relation to the corrosion at its onset, was implemented. Further along, data acquisition hardware and computer software were adapted to the system.

The hypothesis is as follows: The corrosion rate of a metal, under anaerobic conditions, in the presence of

bacteria, is a function of its free corrosion potential and the ability of microorganisms to utilize cathodic hydrogen.

CHAPTER 2
REVIEW OF LITERATURE

Historical Overview

Microorganisms can exist almost anywhere and so can microbial corrosion. In process industries, it can occur inside and outside of equipment. In soil and water, it can happen in aerobic or anaerobic environments.

Microbial corrosion of steel by sulfate reducing bacteria was probably the first area investigated in this field of study and remains one of the most important. In an earlier work (Sifontes and Block, 1991), the authors covered extensively a historical review on the subject. Here some of the main historical aspects are presented here along with a fresh look of microbial corrosion.

Microbial corrosion was first reported before the turn of the century by Garret (1891). He ascribed the corrosion of the lead sheathed cable to the action of bacterial metabolites (ammonia, nitrates, and nitrites). In 1910, Gaines defined the problem more clearly, providing evidence that iron and sulfur bacteria were involved in the corrosion of the inside and the outside of water pipes by making evident the presence of abnormally large quantities of sulfur. He showed that Gallionella, Sphaerotilus, and sulfate reducing bacteria were

responsible for the corrosion of ferrous alloys buried in soil.

In 1924, Bengough and May demonstrated the effect of ammonia produced by bacteria on the corrosion of copper alloys. Later, von Wolzogen Kuhr and van der Vlugt (1934) reported on the anaerobic corrosion of ferrous metals by sulfate reducers. They proposed for the first time a mechanism for microbial corrosion, which actually initiated the systematic studies on microbial corrosion and differentiated the various components of the microbial corrosion process (metal, liquid, and microorganisms). Details on the theory are presented later in this chapter.

Evidence for this bacterially influenced corrosion continued to accumulate from around the world and was reviewed by Starkey and Wright (1945). In 1953, Uhlig reported that the primary role of slime-forming microorganisms was the production of differential aeration and concentration cells type corrosion. The first studies that demonstrated cathodic depolarization with sulfate reducing bacteria was conducted by Booth and Tiller (1960). They added a new dimension to the problem, indicating that depolarization occurred with a hydrogenase positive strain of Desulfovibrio bulgaris and did not occur with a pure strain of hydrogenase negative Desulfovibrio orientis. Furthermore, they demonstrated an additional phenomenon that complicated matters: 1) depolarization was observed when the culture was in active

growth, 2) the stimulation of corrosion was approximately similar for both microbes, 3) the FeS film that formed on the iron samples had an apparent inhibitory effect on corrosion rates, and 4) the corrosion rates reported were much lower than reported rates for similar alloys in natural anaerobic environments in the presence of sulfate reducing bacteria.

Booth and Tiller (1962) and Booth, Robb, and Wakerly (1967) demonstrated also that cathodic depolarization was affected by FeS precipitate, presented experimental evidence that the structure of the FeS film was instrumental in the corrosion process, and indicated that once formed its depolarizing activity continued even in the absence of bacteria.

Since the energy crisis of the 1970s, several reviews on the subject have been published. The results reported by Mara and Williams (1972), King, Miller, and Smith (1973), Smith and Miller (1975), and King, Dittmer, and Miller (1976) suggest that precipitated FeS may initially form a protective film on a ferrous metal surface in the presence of sulfate reducing bacteria. As the microbial corrosion process continues, the film thickens and changes stoichiometrically. As the ratio Fe/S in the film changes from a sulfur deficient to a sulfur rich structure, the film becomes less protective and eventually spalls. Once spalled, the film does not reform and vigorous anodic activity proceeds at the exposed metal surfaces. According to Smith and Miller (1975), the FeS film,

regardless of structure, is cathodic to iron and the corrosion process continues galvanically. Smith (1980) indicated that the FeS film would not remain permanently cathodic in the absence of bacteria. The role of the sulphate reducing bacteria, he suggested, could be either to depolarize the FeS, enabling it to remain cathodic, or to produce more FeS by their metabolism.

Iverson (1981) discounted the FeS argument in his paper. He indicated corrosion rates were above 210 mpy for mild steel specimens exposed to filtered media from actively growing culture of Desulfovibrio (API strain). He also suggested that SRB produced a highly corrosive compound in addition to hydrogen sulfide. The process appeared to depend on whether FeS formed a protective film before the highly corrosive product contacted the metal surface. Thus, it was apparent that a number of factors from the metal and the solution were involved in the process of microbial corrosion by sulphate reducing bacteria.

During the 1980s, researchers found evidence of more factors involved during anaerobic microbial corrosion. Volatile metabolites such as phosphine have been reported to be responsible for microbial corrosion of steels in environments free of sulfate and sulfide (Iverson, 1985). Since iron phosphide was detected among the corrosion products found, it seemed that the amount of the chemical reaction was the result of the competence between the sulfide that would

passivate the metal and the volatile phosphorous (phosphine) that would replace the sulfide. There was no experimental evidence of the chemical nature of the corrosion product, excluding the direct contact between the bacteria and the metal. Postgate (1979) rather concisely accounted for the variety of factors involved in the microbial corrosion process: nature of metal surface, dissolved ions and/or organic matter, biofilm formation, FeS precipitate forms, and other ions (sodium, chlorine) present.

On the other hand, the role of microorganisms in aerobic corrosion was postulated by Olsen and Szaybalski (1949) to be due in part to the formation of tubercles in conjunction with microbial growth, which initiates oxygen concentration cells. This mechanism, along with others, was proposed as the cause of the worldwide problem of microbiologically associated aluminum aircraft wing-tank corrosion that surfaced in the late 1950s and early 1960s. Both commercial and military aircraft were affected. Many microorganisms were reported to be present in significant number in the fuel tank sludge (Churchill, 1963). The same year, Leathen and Kinsel obtained 184 isolates of microorganisms from jet fuel-storage tanks at nine Air Force bases. Results indicated the presence of bacteria and fungi. The predominant bacteria were species of Pseudomonas, and the most prevalent fungi were species Hormodendrum. This case indicated the presence of a variety of organisms including fungi, bacteria, and yeast at a site

of microbial corrosion. The medium was also complex due to the presence of two liquids water and fuel phases.

Later work suggested Cladosporium resinae was the most important fungus encountered in wing tanks and considered it responsible for filter blockage and metal corrosion (Berner and Ahearn, 1977). In 1976, Hill indicated that Cladosporium resinae is only found in subsonic aircraft. In supersonic aircraft, a higher temperature prevails and the predominant flora found were Aspergillus fumigatus, gram negative bacteria, yeast, and other fungi.

Recent evidence appears to indicate that organic acids, produced by fungi, were primarily involved in this corrosion (Miller, 1981).

Other problems due to the activities of SRB have arisen in offshore oil operations (Hamilton and Sanders, 1986). They were identified in the legs and storage cells of offshore structures and include the production of hydrogen sulfide, which is a serious personnel hazard, and the production of bacterial metabolites that give rise to accelerated concrete deterioration (Wilkinson, 1983). Furthermore, internal and external microbial corrosion of long large-capacity subsea pipelines, which transport oil and gas from offshore production fields to shore, are of a major concern due to the high cost associated with their failures and the harm to their surrounding environment (King et al., 1986).

A considerable amount of research on microbial corrosion in relation to sulphate reducing bacteria has been done in the UK, especially in the 1960s. Most of this research was carried out at the National Chemical Laboratory at Teddington (presently the National Physical Laboratory) by a group of well-known researchers in the areas of microbiology and corrosion science. Despite the disbandment of the group in 1968 as a result of a decision of the British Government that microbiology should not be conducted at that institution, the researchers have continued to dominate the literature on microbial corrosion (Tiller, 1985). Among some of the original members of the group are G.H. Booth, D.S. Wakerly, A.K. Tiller, J.R. Postgate, R.A. Kim, W.A. Hamilton, J.A. Hardy, E.C. Hill, and B.N. Herbert.

In the USA, interest in the field has increased in the past 15 years, but the scope has been limited. The most noteworthy efforts began 12 years ago when a microbial corrosion program was initiated by the Materials Technology Group and a separate symposium and technical committee on microbial corrosion was created by the National Association of Corrosion Engineers (NACE) for their annual meetings. Among some of the principal investigators in the USA are D.C. White, R. Mitchell, G. Geesey, W. Characklis, W. Lee, N. Dowling, R. Tatnall, G. Licina, B. Little, and J. LeGall.

During the last 10 years interest in the area has spread in other countries.

Other Aspects of Microbial Corrosion

Despite advancement in the area of microbial corrosion, some researchers have detected some common mistakes in the practice of this type of investigation. Bryant and Laishley (1989, 1993) indicated that the result of uncertain conclusions and overestimates of corrosion due to influence of microorganisms is the failure to account for effects of media constituents such as phosphate. They found that phosphate was one factor among many that together contribute to the overall corrosion rate and that corrosion studies should include controls that account for the constituents of the growth media. There was little or no information on the levels of phosphates on corroding metals. However, sea water contains normally a concentration of 3 μM of phosphate, and dead cells may release some phosphate that may raise the concentration in spacial locations such as crevices and pitts and influence corrosion. Booth and Tiller (1968) did not recommend the use of batch cultures of sulfate reducing bacteria directly to study their effect on the cathodic reaction. They suggested that batch bioreactors were undesirable because the composition of the media would be changing continuously due to the release of metabolites including sulfide, which was known to have a cathodic effect on the corrosion of steel and in the presence of dissolved iron results in the precipitation of FeS.

Hardy (1983) indicated that many attempts of researchers to demonstrate the utilization of cathodic hydrogen by sulphate reducing bacteria and its stimulatory effect on the cathodic reaction have failed because the evidence was based exclusively on measured electrochemical effects. The observed depolarization might have been due to the effect of sulfides present and could not be attributed exclusively to the effect of hydrogenase. He also indicated that there was some evidence that the periplasmic hydrogenase enzyme that appears to be the hydrogen uptake enzyme constituted a greater proportion of the total hydrogenase present in hydrogen-grown cells than of the hydrogenase in lactate-grown cells. This feature, as explained earlier, is a characteristic of bacteria adaptation that is seldom considered during microbial corrosion research. Researchers most of the time use bacteria that have been cultured in standard media rather than wild type cultures. Wild type species of Desulfovibrio have been shown to lose their adhesion properties when they are transferred from the wild to laboratory and grow in standard medium.

Rajagopal and LeGall (1989) suggested that the ability of some sulphate reducing bacteria to reduce nitrate or nitrite with hydrogen as sole energy source provides a better experimental system to study the cathodic depolarization phenomenon, since problems with measurements of dissolved iron

and growth in the presence of precipitated sulfides resulting from the customary sulphate reduction can be avoided.

The presence of organic electron donors has been considered during cathodic depolarization of steel. Widdel (1988) indicated that most Desulfovibrio spp are able to grow on acetate and carbon dioxide as carbon sources and hydrogen as sole energy source. Cord-Ruwisch and Widdel (1986) found that the availability of organic electron donors appears to be an important factor that influences the removal of cathodic hydrogen. If a favorable organic energy source was present, sulphide was produced, indicating that iron alone did not allow sulphate reduction. They suggested either that cathodic hydrogen was preferentially oxidized with the organic substrate or that sulphide from sulphate reduction with the lactate reacts with the remaining ferrous ions or ferrous hydroxide from the corrosion process to produce more FeS.

In addition to the above observations, some other important scientific areas related to the microbial corrosion phenomenon need to be addressed in order to envision the complexity of the problem. Those areas include bacterial adhesion, surface thermodynamics, interfacial chemistry, hydrogen embrittlement, interspecies hydrogen, bioenergetics, microbial ecology, and applied fundamentals of metallurgy, electrochemistry and chemistry. Information on those subjects is referred to in the cited literature. Specific topics include the prevailing mass transfer conditions and the

surface properties of the phases involved, which include molecular fluctuations at the electrical double layer (Liu, 1983), the adhering bacteria, the substrate, and the suspended media (Absolom et al., 1983; David and Misra, 1985), chemical interaction at metal surfaces and at the liquid interface (Sato, 1987), and metallurgical aspects such as microstructure, grain boundaries, inclusions, surface contamination, and sources of corrosion currents (Godard, 1980; Mears and Brown, 1941).

Microorganisms Involved in Microbial Corrosion

The microorganisms that have been associated with microbial corrosion include many genera and species. Microorganisms are anatomically simple yet biochemically complex. They may be divided into three groups: (1) algae and fungi, (2) protozoa, and (3) monera (e.g., bacteria). Many of these organisms have been firmly established in laboratories and field sites as having roles in the corrosion process, whereas others have merely been isolated from suspected corrosion sites. Microorganisms are sustained by chemical reactions by ingesting reactants and eliminating waste products. Thus these processes can influence corrosion generaly by 1) directly influencing anodic and cathodic reactions, 2) influencing protective surface films,

3) producing deposits, 4) producing corrosive metabolites, and 5) feeding on corrosion inhibitors.

Several characteristics of microorganisms enhance their involvement in corrosion. They are generally very small, starting from less than 0.2 μm, which allows them to penetrate crevices very easily, to several hundred μm in length. Some of them are motile, which aids in their migration to more favorable environmental conditions. Microorganisms are able to establish in sites that encourage their growth. For instance, microbes establish and colonize surfaces in cooling water systems where food sources concentrate at metal surfaces because of their hydrophobic properties. They can withstand a wide range of environmental conditions: pH values from 0 to 11 (Brock and Madigan, 1991), temperatures from -30 to 110°C, and oxygen concentrations from 0 to 100% (Morgan and Dow, 1986).

Microorganisms can adhere to a surface and form colonies of different species. These consortia, once formed, can sustain survival under adverse conditions. They can reproduce themselves to a great number in a short time. This fact allows them to bloom and take over an environment quickly. They are easily dispersed in air, water, animals, etc., and adapt to other environments in which it may be easier for them to grow. Many can adapt easily to a wide variety of substrates, such as the Pseudomonas, some species of which can use well over 100 different kinds of food as sole carbon

and energy sources. Many can produce extracellular polysaccharides or slime layers where a consortia of bacteria can develop and consequently influence corrosion (White et al., 1986). These layers attract food and other microorganisms and cause several other well-known problems in the process industry, such as poor heat transfer.

Some microbes produce spores that resist the most severe environmental conditions and are capable of surviving for long periods of time by remaining dormant. They can quickly colonize surfaces when the environment changes to their liking. Some resist antimicrobial agents by virtue of their ability to degrade them or by resisting permeability through the cell wall or by the extracellular polysaccharide protection. Such resistance may be acquired by mutation or acquisition of a plasmid. Many species produce a wide variety of organic acids that may promote corrosion of many alloys even at low concentrations (e.g., Clostridium acetobutylicum produces acetic acid).

Some species produce mineral acids that are extremely corrosive. Thiobacillus thiooxidans produces sulfuric acid, which is of economic importance in biohydrometallurgy because it makes possible the leaching of metal sulfide ores. In the case of pyrite, it oxidizes both the sulfur and ferrous moiety. Boes and Kuenen (1983) have recently reviewed the sulfur oxidizing bacteria and their relationship to corrosion and leaching. Several bacteria metabolize nitrate, sometimes

used as a corrosion inhibitor (e.g., Pseudomonas spp reduce nitrate and nitrite to nitrogen gas). Other organisms convert nitrate to nitrite or ammonia to nitrite (e.g., Nitrosomonas) and others turn nitrite into nitrate (e.g., Nitrobacter). Many organisms form ammonia from the metabolism of amino acids. This forms ammonia ions in basic solution, which may be corrosive to copper alloys.

Microorganisms such as sulfate reducing bacteria produce enzymes, which may be excreted outside the cell and which can act on substances outside the cells. For instance, hydrogenase has been reported as being responsible for depolarizing cathodic sites during the microbial corrosion of iron and steel. Many organisms can produce carbon dioxide and hydrogen as a result of their fermentative metabolism. Carbon dioxide in acidic solutions becomes carbonic acid, which is highly corrosive, and hydrogen can polarize metal surfaces of stainless steel and may cause hydrogen embrittlement.

Many genera of bacteria that normally use organic compounds as carbon and energy sources can use hydrogen gas as their energy source and carbon dioxide as their carbon source and live chemoautotrophically. This can cause depolarization of cathodic sites on steel and promote corrosion (e.g., Methanogens).

Some bacteria can oxidize or reduce metals or metallic ions directly. For example, the iron-oxidizing bacteria (Gallionella, Sphaerotilus) oxidize ferrous ion to ferric ion.

The ferric compounds precipitate in a sheath around the cells and form tubercles in pipes and cause plugging. Thus, concentration of cells is easily formed under those deposits. On the other hand, ferric ion can be reduced to ferrous ion by Pseudomonas spp from oil wells and marine sediments. It has been suggested that the ferric film, which normally stabilizes the surface of mild steel from corrosion, are destroyed leaving the surface susceptible to corrosion attack. Other bacteria can oxidize or reduce metals such as manganese.

Microorganisms can form synergistic communities (e.g., algae and bacteria). These consortia can accomplish things that individually would be difficult if not impossible, such as the case of fungi and Desulfovibrio spp.. The fungi break down wood to organic acids and consume oxygen, thus providing the food and anaerobic conditions for Desulfovibrio spp.. Communities providing protection for individuals can also change structure, dominant species, etc. by genetic mutation, and can adapt to environmental changes, even to deliberate chemical changes intended to kill them. More details on this subject may be found in Sifontes and Block (1991).

Mechanisms of Microbial Corrosion

For proper selection of methods to prevent or control microbial corrosion, it is necessary to know the mechanisms by which microbial activity affect the deterioration of metals.

The mechanisms of microbial corrosion in some cases are well defined, but where environments encourage the activity of bacteria, the corrosion processes are more complex and still not fully understood. The mechanisms of microbial corrosion can be subdivided in direct and indirect mechanisms. If a microbe interlinks an electrode process with its own metabolism or presence, it is a direct effect (i.e., differential aeration cell produced by sessile bacteria), otherwise it is an indirect effect (i.e., corrosive metabolites produced by planktonic bacteria).

Even though the mechanisms of microbial corrosion are not well understood, microbes that cause or influence corrosion have been classified previously (Kobrim, 1976) into the following groups:

1) Acid production. Some microbes can oxidize sulfur compounds to sulfuric acid. Very low pH has been reported in places where sulfur oxidizing bacteria are active. Many species produce a wide variety of organic acids (e.g. acetic, butyric, succinic, and formic) which may promote corrosion of many metals and their alloys.

2) Protective coating destruction. Protective coatings ranging from polymeric materials to passive films can be broken by the activity of microorganisms and corrosion of bare metal starts rapidly.

3) Production of corrosion cells. Differential aeration and ion concentration cells are notable examples such as the case

of a metallic surface that is accessible to oxygen. If a deposit such as a biofilm or a corrosion product covers it, the surface under the deposit is shielded from oxygen and the surface outside of the deposit is not. This results in a corrosion cell.

4) Sulfur reduction and oxidation. Sulfate reducing bacteria are the most publicized class of corrosive microbes. They reduce sulfates to sulfides and can depolarize cathodic sites by consuming hydrogen. Most sulfur oxidizing bacteria known fall in the category of acid producers.

5) Concentration of anions and/or cations. Iron and manganese bacteria are examples of this category. They generally form thick, bulky deposits which create concentration cells or harbor other corrosive microbes. This group is also known as metal ion oxidizers or reducers.

6) Hydrocarbon utilization. Certain microorganisms have been observed that destroy organic coatings or linings in the presence of hydrocarbon fuels. Some others destroy metals such as aluminum and feed on hydrocarbon fuels.

7) Slime formation. Certain algae, yeast, bacteria, and fungi may form deposits which foul heat transfer equipment and produce concentration cells on metal surfaces.

The above mechanisms can be grouped in three general modes of microbial attack based on their metabolism: corroded material serves as substrate for microbial growth, microbes

colonize material surface but feed on something else, and microbes produce metabolites that corrode material.

Theory of Cathodic Depolarization

The theory of cathodic depolarization was postulated in 1934 by von Walzogen Kuhr and van der Vlught. The idea of the authors came from their interpretation from the electrochemical point of view of the anaerobic corrosion of a cast iron pipe in wet soils near Amsterdam. Since the pipe was under clay at a near neutral pH, the severly corroded pipe did not allow them to explain the phenomena using the reduction of oxygen as the cathodic reaction. The alternative cathodic reaction was the reduction of hydrogen that was feasible under those conditions.

The theory proposes that when iron is immersed in water, a natural equilibrium is set up between the ferrous cations released at the anode, and the metal surface negatively charged at the cathode by the remaining electrons. The dissolving process continues only if the electrons are removed. Under aerobic conditions oxygen serves as an electron acceptor resulting in rust formation. Under anaerobic conditions free protons from the dissociation of water are reduced on the cathodic metallic surface by the remaining electrons, to form a protective hydrogen polarized envelope that protects the iron metal from further

dissociation. A dynamic equilibrium is established which keeps the iron polarized. The theory suggests that the principle mechanism of anaerobic corrosion is cathodic depolarization of the iron surface by hydrogen oxidizing microorganisms such as sulfate reducing bacteria and methanogen. These organisms disturb the equilibrium by oxidation of the cathodically formed hydrogen with sulphate and carbon dioxide as electron acceptors respectively, via the hydrogenase enzyme. In an attempt to re-establish the anodic/cathodic equilibrium more iron is oxidized, the end result of which is pitting formation.

Table 2-1. Chemistry of the cathodic depolarization theory.

component	reaction
1)Metal dissolution	$4Fe \rightarrow 4Fe^{+2} + 8e^{-}$
2)Hydrogen reduction	$8H^{+} + 8e^{-} \rightarrow 8H \rightarrow 4H_2$
3)Water dissociation	$8H_2O \rightarrow 8H^{+} + 8OH^{-}$
4)Microbial activity	$SO_4^{-2} + 8H \rightarrow S^{-2} + 4H_2O$
5)Corrosion product	$Fe^{+2} + S^{-2} \rightarrow FeS$
6)Corrosion product	$3Fe^{+2} + 6OH^{-} \rightarrow 3Fe(OH)_2$
7)Total rxn	$4Fe + SO_4^{-2} + 4H_2O \rightarrow 3Fe(OH)_2 + FeS + 2(OH)^{-}$

The importance of this theory lies in the fact that it separates for the first time the three components of the

microbial corrosion system which include the microorganisms, the metal, and the suspended medium.

A large volume of literature up to the last decade has been influenced by the theory of cathodic depolarization and has referred to it, either to prove or to disprove it. However, the use of electrochemical techniques allowed Horvath and Solti (1959) to discover an anodic effect in addition to the cathodic effect of the theory. They studied this effect as a function of pH, environmental regulatory conditions and the concentration of FeS present. According to those results, SRB had an indirect role, which would be the stabilization of the sulfide compounds over the metal surface by modification of the redox potential.

One of the most conclusive findings was reported by Costello (1974) who indicated a cathodic effect due to the hydrogen sulfide produced by the sulfate reducing bacteria, suggesting that hydrogen utilization by the sulfate reducing bacteria became secondary and so the participation of those bacteria in the corrosion process. Furthermore, It has been suggested as an amendment to the corrosion theory that solid ferrous sulfide, in contact with iron, acts as a cathode (Booth et al., 1968). Other authors proposed that reduced phosphorous compounds, too, are involved in the anaerobic corrosion process (Iverson and Olson, 1984). Recently it was reported that phosphate and hydrogenase can affect the corrosion of mild steel. Phosphate reacts on mild steel with

concomitant production of hydrogen gas and the formation of an iron/phosphate complex (vivianite) Bryant and Laishley, 1990; Weimer, et al., 1988). The enzyme hydrogenase was reported to accelerate cathodic depolarization by oxidizing the hydrogen produced (Bryant and Laishley, 1993). Although many studies have clearly demonstrated the involvement of sulphate reducing bacteria and methanogens in corrosion, only a few have shown the influence of those microorganisms using the electrons from the metal as energy source.

On the other hand, some authors have provided evidence in support of the cathodic depolarization theory (Cord-Ruwisch and Widdel, 1986; Hardy, 1983; Tiller and Booth, 1962; Booth and Tiller, 1960, 1962). Few, however have actually demonstrated that this phenomenon is coupled to microbial growth (Belay and Daniels, 1990; Rajagopal and LeGall, 1989; Daniels et al., 1987; Pankhania et al., 1986; and Tomei and Mitchell, 1986). In conclusion, research indicates that the described theoretical mechanism is not entirely correct.

Previous Studies in Microbial Corrosion

Despite the large amount of literature in microbial corrosion, it is still not an easy subject to understand, because of the multiplicity of factors at play. Some authors have indicated that microbial corrosion is a newly discovered problem or an emerging science, possibly still in its infancy

(Tatnall, 1988). Others have confirmed by surveys that the continued incidence of microbial corrosion could be due to a general lack of awareness of the problem (Wakerly, 1979). The fact is that its scientific study began more than 80 years ago (Tiller, 1982) and after the work of von Wolzogen Kuhr and van der Vlugt in 1934, who established the classical mechanism of anaerobic corrosion, the subject of microbial corrosion in general and of anaerobic corrosion in particular has become recognized of prime importance (Bessems, 1983; Hamilton, 1985).

Most of the literature on microbial corrosion, before 1960, was concerned basically with observations of the effect of bacteria on the environment. During the 1960s, there was considerable activity on the field and most of the research was focused on gaining understanding at the different mechanisms. Some notable papers of this period belong to Postgate (1960), Horvath (1960), Sorokin (1966), Iverson (1966), Booth, Elford and Wakerly (1968), Booth and Tiller (1968), and Costello (1969).

During the 1970s the interest in the subject broadened the scope of research involving scientists from other fields including plant engineers, corrosion scientists, microbiologists, and biochemists (Sequeira and Carrasquinho, 1988). This has brought improved understanding of the ecology, nutrition and physiological requirements of the microbes involved in microbial corrosion, which also improved

the biochemical techniques used in both the field and the laboratory. In addition, the publication of case histories of a diverse range of failed industrial equipment has enhance understanding of the problem. This period is well described in the articles by Iverson (1972), King and Miller (1973), Mara and Williams (1972), Miller and King (1975), Kobrin (1976), Widdel and Pfennig (1977), Jorgensen (1977, 1978, 1980), Wakerly (1979), and Postgate (1979).

It was only within the last decade that microbial corrosion was recognized as a serious problem in the chemical industry by the leading corrosion society in the world (NACE) and substantial advancement has been achieved specially in the areas of microbiology and corrosion. In microbiology, among other findings, the biochemistry of dissimilatory sulfate reduction in Desulfovibrio has revealed enzymes and electron carriers of special character and structure whose function and distribution within the cell are just beginning to be revealed, indicating new discoveries in the peculiar energy generation systems in these bacteria.

In corrosion, sophisticated methods to study it, have evolved; such as new methods in electrochemistry, metallography, macroanalysis, and microanalysis. A significant part of the research in that period is described in the articles by Hamilton (1985), Iverson (1987), White et. al. (1986), Tatnal (1981), Odom and Peck (1984), Cord-Ruwisch and Widdel (1986), Tiller (1982), Miller (1981), Hardy (1981,

1983), Rajagopal and LeGall (1989), Bryant and Laishley (1989), Daniels et al. (1987), and Widdel and Pfennig (1981).

Today, microbial corrosion is well recognized as a serious problem in most industries, particularly in oil and gas, the power generating and the process industries. In the chemical industry alone, multimillion dollar failures due to microbial corrosion have been reported involving cooling water systems (Felzin et al., 1988).

While the above studies have yielded a wealth of information, the literature reveals that the corrosion community still knows very little about how microorganisms influence or induce corrosion, and what the role of microorganisms is in the corrosion process. In general, most of the research deals with observations related to the basic problem, case histories, biological research, and reviews of literature. It is important to notice that this was not usually the fault of the investigator; in the majority of instances the specialist lacked interdisciplinary help required to tackle such complex studies.

Microbial corrosion is by definition an interdisciplinary field that requires among others, the understanding of microbiology, corrosion science, metallurgy, electrochemistry, transport phenomena, and surface chemistry. Several factors have cause a further hindrance in the development of this microbial corrosion. Among some of the factors are: the lack of awareness; the difficulty of growing, isolating, and

identifying anaerobic microorganisms; the adaptation of techniques from clinical microbiology to handle the microbes involved; the fact that to the average researcher microbial corrosion spans the boundary of traditional specialties; and the idea that many microbiologists have of microbial corrosion as a rather specialized even esoteric field, and the lack of acceptance of the role of bacteria in corrosion by corrosion engineers.

Actually, many theories on how microorganisms influence or induce corrosion have been proposed. However, none of them are fully proven but rather equal number of existing papers on the subject claim to support or refute these theories. Consequently, it seems fair to say that there is little uniform agreement among those working on this field about what is really going on (Tatnall, 1988).

Customary practices, used during the investigation of microbial corrosion, include separated studies of either the microorganisms, the metal or the fluid but ignore their interaction. In addition, most laboratory microbial corrosion experiments use flowthrough type bioreactors which are run for weeks or even months. The most common corrosion measurement methods used are adopted from classical corrosion science such as Tafel plots and weight loss techniques. In many cases, these practices have failed to give reproducible results because of the length of the experiments, nature of the metal surface, and fluid chemistry in addition to microbial

ecological considerations of such a complex systems. The application of external potentials to the system, a common practice in microbial corrosion experiments, may produce significant thermodynamic changes known to affect the stability of the system, and constitute a source of uncontrolled variables.

The practical separation of the three components of the microbial corrosion systems as suggested by the cathodic depolarization theory, and the work performed by Daniels et. al. (1987), set the stage for studying the microbial utilization of cathodic hydrogen during corrosion. If hydrogen oxidizing bacteria can take up the hydrogen produced cathodically from steel in well defined experimental systems then there exists the possibility to further explore in this field and increase our understanding of these microbes and their mechanisms of attack on metals.

Microbial Corrosion Control

Prior to 1970 the control of microbial corrosion focused on the use of cathodic protection and antimicrobial agents. This practice was complemented by improvements in surface coatings, in particular antifouling systems such as tapes and wrappings. Because of the limited ability of those methods to control bacterial activity and the inefficacy of the methodology to asses antimicrobial agents; the main driving

force after 1975 has been the consolidation of several methodologies and assessment procedures to improve the standard practice (Tiller, 1985).

In 1964, Saleh and co-workers evaluated nearly 200 antimicrobial agents and concluded that laboratory evaluations of those chemicals can only be considered as an introductory sign and should be supplemented with trials on the field. In 1983 Bessems recommended the importance of assessment procedures and Gaylarde and Johnston emphasized the deficiencies in current methodology and the need for improvement. During last decade a joint venture of the Institute of Corrosion Science and Technology and the National Association of Corrosion Engineers has reviewed and updated the current recommended practice for monitoring bacterial growth. Topics such as killing time, the importance between planktonic and sessile bacterial consortia and their attachment properties are now important issues in the control of microbial corrosion (Tiller, 1985).

Due to the complexity of the environmental factors that results in microbial corrosion, the success of a control program depends more in our outlook on the available information about the problem, and becomes a challenge to identify the most practical and economic solution.

In general, the control of microbial corrosion requires a sound strategy. This involves a diagnosis of the microbial corrosion problem that begin with determining the cause and

the mechanisms associated with the corrosion problem. A sound diagnosis include: comprehensive current system diagrams, materials of construction, fabrication methods, operating history, chemical analysis, site specific environmental conditions, biological history, and historical and current chemical treatment. Unfortunately, if this is not followed, adverse consequences may result. For example, a strong oxidant may have a good killing power but may produce severe local corrosion if applied to a particular system.

Fellers (1989) suggested that the best strategy is the product of a multi-disciplinary team which looks at all aspects of the problem, examines root cases, and objectively evaluates alternatives. He also indicated in a later paper that if the main strategy is to maintain the system clean of microbial growth, instead of looking for independent solutions to several problems, several issues may be avoided including microbial corrosion (Fellers, 1990).

The essential strategies to control microbial corrosion include: detection, prevention, and mitigation. Detection techniques are basic to prevention and mitigation. They include electrochemical methods, microbiological methods, physical and metallurgical methods, in system monitoring techniques, and other laboratory techniques. Details on these techniques are found in earlier work of the author (Sifontes and Block, 1991). Additional detection techniques include visual inspections to identify suspected indications of

microbial attack and side stream monitors for determination of corrosion and fouling rates.

Prevention and mitigation techniques hold the highest payback in plant protection and preservation. There is no universal approach to the prevention and mitigation of microbial corrosion since it is almost impossible to use a single preventive method. The prevention and mitigation techniques offered by the current technology include planning considerations such as material selection, design, and non-metallic materials; physical-chemical control such as corrosion inhibitors, selection and control of the environment, protective coatings, electrochemical protection, maintenance cleaning, and chemical control; and biological control such as antimicrobial agents, and environmental system control. Details on above prevention techniques are found in earlier work of the author (Sifontes and Block, 1991). Other prevention and mitigation techniques include general corrosion control such as prevention of scales which enhances biofouling, suspended solids dispersion such as the use of penetrants of other surface active agents which re-disperse fouling materials (Fellers, 1990).

Finally, an effective control of microbial corrosion involves a combination of selected techniques to solve an specific problem. Numerous history cases that include the results of the applied techniques are available throughout the literature cited. A reported case history of successful

corrosion control in a cooling water system used a combination of biodispersant/biocide. It employed an organic corrosion inhibitor, a polyacrylate/phosphonate dispersant, and a combination of two microbiocides used simultaneously (Honneysett et al., 1985).

the use of surfactants (penetrants /biodispersants) has improved the effectiveness of microbial corrosion control. Chemically, these substances are composed of nontoxic organic compounds with penetrating and dispersing properties. The biodispersants allow the sessile colonies to be penetrated by the antimicrobial agents, thus used at lower dosages with improved effectiveness. They inhibit the biomass produced from becoming so massive that antimicrobial agents can not penetrate the consortia of microbes. Some penetrants are hydrophobic to the extent that a film forms on the metal surfaces allowing less deposition of sessile colonies associate or not with microbial corrosion.

Other references that contain a variety of history cases include: Microbial Corrosion proceedings by the Metals Society of London, 1983; International Conference on Biologically Induced Corrosion proceedings by the NACE, 1985; Microbial Corrosion 1 proceedings by the First European Federation of Corrosion workshop on Microbial Corrosion, 1988; and Microbially Influenced Corrosion and Biodeterioration proceedings by the International Congress on Microbially Influenced Corrosion, 1990.

Research Approach

The following tasks were considered in order to comply with the objective of this research. The first task included the evaluation of some new experimental flowthrough and batch bioreactors to reproduce and study the anaerobic microbial corrosion phenomena. The initial task consisted of runs of bioreactors with the object of reproducing microbial corrosion in the laboratory. The second task involved the use of the batch bioreactor to study the onset of microbial corrosion, using conditions that resemble the inside of gas transmission pipelines. The operation of the batch bioreactor was examined concerning practical functioning for head space composition, sterilization method, reducing agent use, metal coupon preparation, and bacteria handling. Different metals and their alloys were tested for electroactivity and their ability to produce hydrogen; and different hydrogen oxidizing bacteria were also tested to determine their ability to uptake hydrogen. A third task involved the development of an experimental system that included the redesign of the batch bioreactor, development of a working electrode preparation of bacterial suspensions, preparation of mineral solution electrolyte, and the implementation of a data acquisition system to accommodate electrochemical measurements, in an attempt to quantify the biological component of corrosion. The redesign of the batch bioreactor consisted of improvements

to the experimental system that resulted in the development of the single, dual, and triad flask electrochemical cells. A forth task involved the general setting of parameters to assure simpler and reliable results such as the selection and setting of fixed conditions and analytical procedures.

CHAPTER 3
MATERIALS AND METHODS

Introduction

The overall objective of this research was to study anaerobic microbial corrosion at its on-set and to quantify cathodic hydrogen utilization by bacteria. For this purpose, a total of seven experimental set-ups were utilized. These include: the 1) flowthrough bioreactor, 2) batch bioreactor, 3) single flask electrochemical cell or single cell, 4) dual flask electrochemical cell or dual cell, 5) triad flask electrochemical cell or triad cell, 6) artificial hydrogen uptake triad cell, and 7) galvanic couple triad cell.

Initially, the flowthrough bioreactor was used to replicate microbial corrosion and to get familiar with the laboratory techniques and analyses required for the investigation. This traditional bioreactor design used a known bacteria triculture commonly found at microbial corrosion sites and a glucose lactate yeast extract (GLYE) medium that allowed the three bacteria to grow rapidly. Runs of this bioreactor were performed using carbon steel coupons in the presence of the bacteria triculture in GLYE medium. Then, since the corrosion reaction and the biofilm formation developed relatively fast in the flowthrough bioreactor, a

simpler batch bioreactor to study the on-set of the reaction was used. This new bioreactor offered the best alternative to study the initiation of the microbial corrosion process and was able to reproduce microbial corrosion of carbon steel within 24 hours in GLYE and in mineral solution media. The batch bioreactor was redesigned and instrumented to include electrochemical measurements and became the single flask electrochemical cell. The single cell was used to measure free corrosion potential in the presence and absence of bacteria. It was improved later to the dual cell and the triad cell. The dual cell allowed bacteria to avoid direct contact with toxic metals and the triad cell provided for measurement of a differential corrosion current. The last two experimental set-ups were identical to the triad cell, except they were used to study the effect of an artificial hydrogen uptake and to determine the reliability of the electrochemical measuring system respectively.

All electrochemical set-ups used the mineral electrolyte solution developed for the purpose of studying microbial corrosion at its on-set and hinder the formation of a biofilm

Flowthrough Bioreactor

Description

The 3-L flowthrough bioreactor system, used for the preliminary work of this research was designed in house and

built out of 5 cm ϕ PVC pipe components. It consisted of two sections for the study of sessile and planktonic bacteria activity and their effect on metal deterioration. A basic diagram showing its components is illustrated in Figure 3-1.

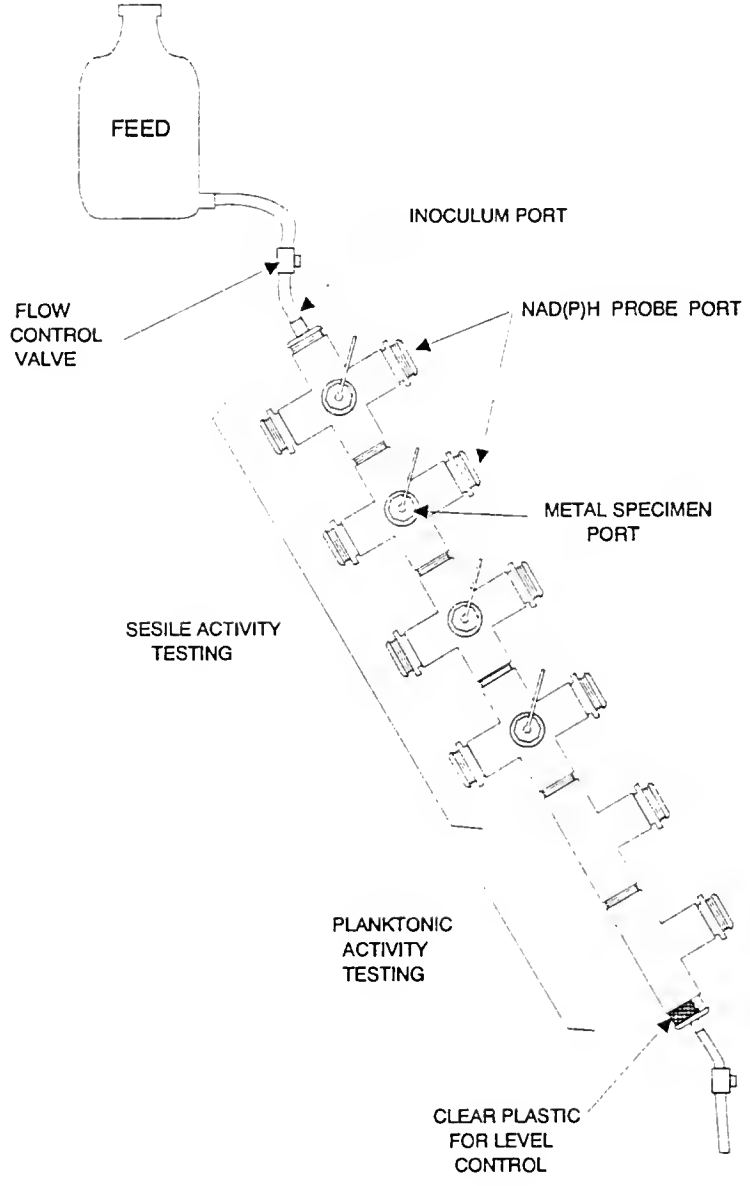


Figure 3-1. Flowthrough bioreactor general description of components

The first section, for sessile activity testing, consisted of eight side screw cap ports that fit a fluorescence probe, developed by Biochem Technology, Inc., King of Prussia, Pennsylvania, and four top screw caps, to which metal coupons and reference electrodes are attached. The second section, for planktonic activity testing, contained two side screw caps ports for attachment of the fluorescence probe, and a clear section at the end of the reactor for the purpose of controlling the bioreactor head space volume.

The bioreactor was fed from a 4-L stoppered flask through a tubing, Tygon R-3603, and an effluent flow of 125 mL/hr was controlled using a hose clamp on a 30 cm tubing, Tygon R-3603, at its outlet.

A known bacteria triculture composed of Entrobacter aergenes, Desulfovibrio desulfuricans, and Clostridium acetobutylicum were used as the inoculum. Bacteria were obtained from the American Type Culture Collection (ATCC).

Steel coupons, 5 mm x 20 mm x 10 mm, polished to 240 grid, and tied plastic strips to a 1/4 " ϕ teflon rods were attached to the upper screw caps of section one. Figures 3-1b and 3-1c illustrate the assembly combinations of metal coupon/fluorescence probe and a metal coupon/fluorescence probe/reference electrode, respectively. Figure 3-1d shows an actual set-up of the flowthrough bioreactor.

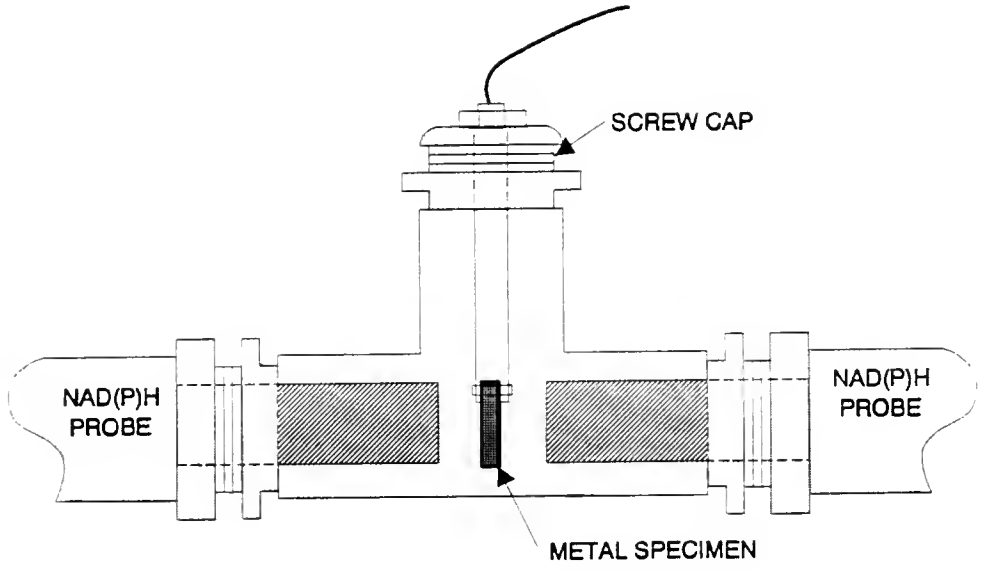


Figure 3-2. Flowthrough bioreactor metal coupon and fluorescence probe setup.

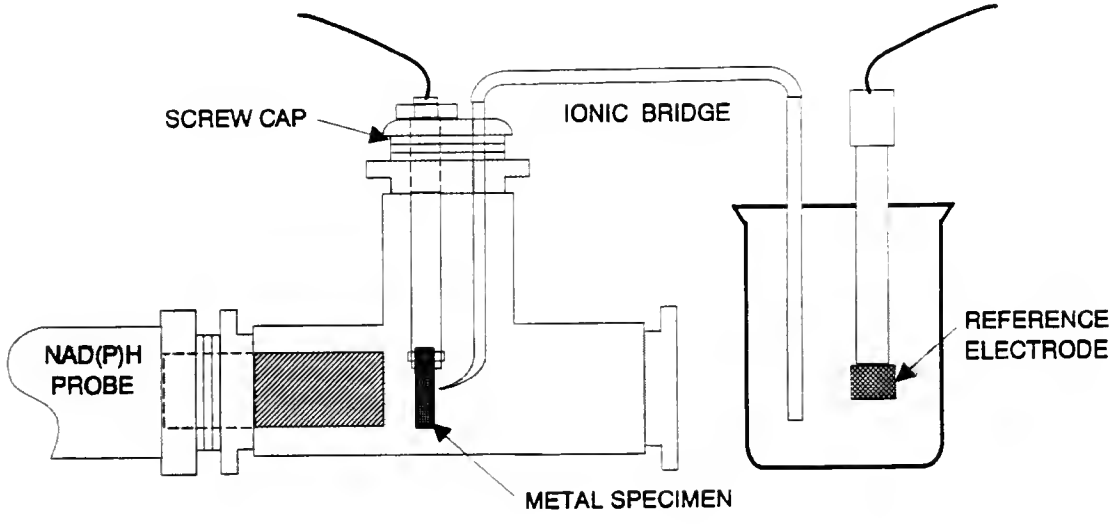


Figure 3-3. Flowthrough bioreactor metal coupon, fluorescence probe and reference electrode setup.

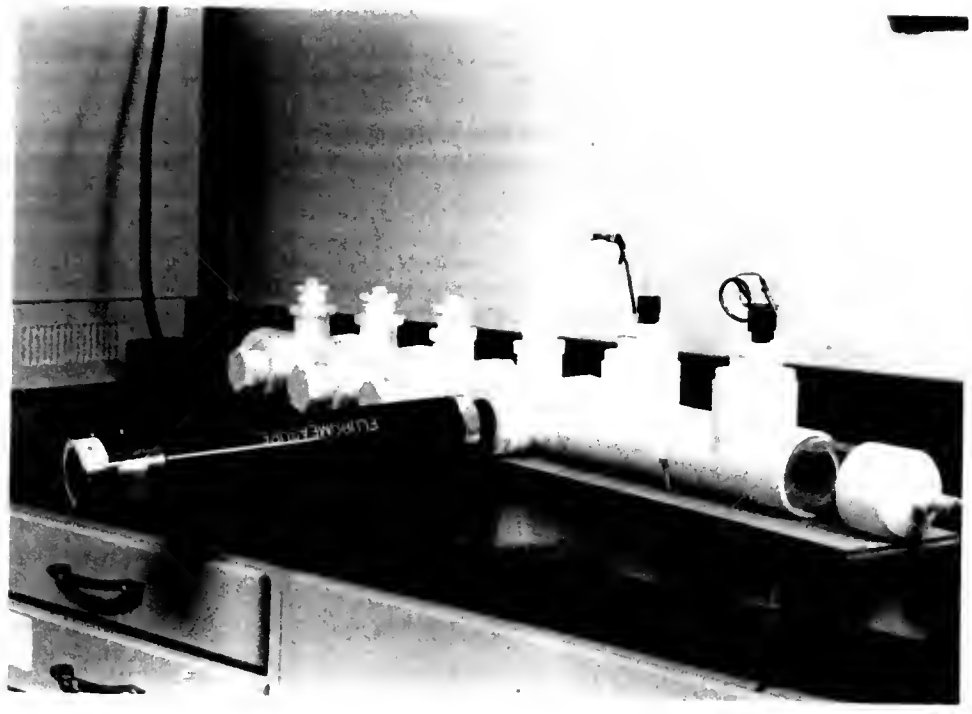


Figure 3-4. Flowthrough bioreactor components.

Operation

The flowthrough bioreactor system was operated at a 1-day retention time (125 mL/hr). Medium was fed to the bioreactor by gravity from the 4-L reservoir under a nitrogen atmosphere to assure anoxic conditions. The feed, a glucose-lactate yeast-extract (GLYE) media, was prepared fresh daily, sterilized and neutralized prior to use.

Effluent samples for pH and volatile fatty acids were taken directly from the effluent. Metal coupons samples for scanning electron microscopy analysis were sampled under aseptic conditions while sparging with N_2 gas and placed

immediately into 10 mL vials containing a 0.5% glutaraldehyde, 2% formaldehyde, cacodylate buffer, pH 7.2. Metal samples were further processed, according to protocol described in Table 3-1, for scanning electron microscopy examinations.

Table 3-1. Protocol for metallic surface fixation for scanning electron microscopy examinations.

1) Place metal coupon in a 0.5% glutaraldehyde, 2.0% formaldehyde, cacodylate buffer, pH 7.2 solution for 5 min at room temperature, then on ice for 10 min.
2) Wash coupon in ice-cold buffer two times for 5 min each.
3) Wash coupon in ice-cold ethanol solutions for 5 min each, in the following ethanol concentrations: 25%, 50%, 75%, and 95%.
4) Wash coupon at room temperature in 100% ethanol.
5) Wash coupon in acetone for 15 min at room temperature.
6) Wash coupon in fresh acetone at room temperature for 30 min.
7) Treat coupon with solutions of epon-araldite 30%, 70%, and 100% for 1 hr each at room temperature, except the 100% which is done at 60°C in oven.

Bacteria inoculations were done as follows for initial run. Ten mL of E. aerogenes were inoculated first in order to reduce the media. After 24 hours, 15 mL each of D. desulfuricans and C. acetobutylicum were inoculated. For

other runs, bacteria were inoculated within six hours in amounts 10 times larger than the volumes used in the initial run. All runs were temperature controlled at 35°C inside a walk in incubator.

GLYE media was prepared using ingredients outlined in Table 3-2, Table 3-3, and Table 3-4. Media was prepared in 4-L feed reservoir flasks and autoclaved at 15 psig for 15 min (Balch et al., 1979).

Table 3-2. Glucose Lactate Yeast Extract Medium Composition.

Yeast Extract	0.5 g
Glucose	0.5 g
Sodium Lactate	0.5 g
Mineral Solution 1 (table 3)	25.0 mL
Mineral Solution 2 (table 3)	25.0 mL
Trace Mineral Solution 3 (table 3)	5.0 mL
Trace Vitamins Solution 4 (table 2)	5.0 mL
L-Cysteine $HCl \cdot H_2O$	0.5 g
$Na_2S \cdot 9H_2O$	0.5 g
Resazurin (0.5 mg/mL)	1.0 mL

Complete to 1-L with distilled deionized water, adjust pH to 7.5 with 5N NaOH and autoclave for 15 min at 15 psig.

Table 3-3. Trace Vitamins Solution 4 Composition.

Biotin	2 mg
Folic Acid	2 mg
Pyridoxine HCl	10 mg
Thiamine HCl	5 mg
Riboflavin	5 mg
Nicotinic Acid	5 mg
DL-Calcium Pantothenate	5 mg
Vitamin B12	0.1 mg
p-Amino Benzoic Acid	5 mg
Lipoic Acid	5 mg

Complete with distilled and deionized water to 1-L

Table 3-4. Mineral Solutions Composition.

1) Mineral solution 1

K_2HPO_4	6.0 g/L

2) Mineral solution 2

KH_2PO_4	6.0 g/L
$(NH_4)_2SO_4$	6.0 g/L
NaCl	2.0 g/L
$MgSO_4 \cdot 7H_2O$	2.6 g/L
$CaCl_2 \cdot 2H_2O$	0.16 g/L

3) Trace Minerals solution 3

$N(CH_2CO_2H)_3$	1.5 g/L
$MgSO_4 \cdot 7H_2O$	3.0 g/L
$MnSO_4 \cdot 2H_2O$	0.5 g/L
NaCl	1.0 g/L
$FeSO_4 \cdot 7H_2O$	0.1 g/L
$CoSO_4$	0.1 g/L
$CaCl_2 \cdot 2H_2O$	0.1 g/L
$ZnSO_4$	0.1 g/L
$CuSO_4 \cdot 5H_2O$	0.01 g/L
$AlK(SO_4)_2$	0.01 g/L
H_3Bo	0.01 g/L
$Na_2MoO_4 \cdot 2H_2O$	0.01 g/L

Batch Bioreactor

Description

The batch bioreactor system consists of a 250 mL bioassay bottle with the metal coupon suspended in 150 mL of liquid media by a nylon string attached to a crimp top butyl rubber septum to preserve bottle anaerobically. A typical batch bioreactor experiment is shown in Figure 3-2. This set-up was used to study microbial corrosion under a known growth environment, medium, metal coupon, and head space, using different bacteria combinations.

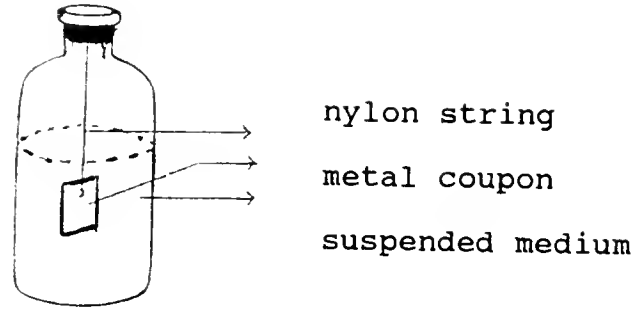


Figure 3-5. Batch bioreactor.

Prior to the tests, the batch bioreactor was assembled using a 250 mL bioassay bottle to which 150 mL of GLYE media was added and a metal coupon is suspended in the media. The metal coupon was fabricated from ASTM-ASME SA106 grade B1 steel pipe 2.5 cm ϕ, schedule 80, obtained from Texas Eastern, Louisiana. Coupons were machine ground to a size of 1.5 cm x 2.0 cm x 3 mm with a side hole of 3mm ϕ for the attachment of the nylon string that holds them to the butyl rubber stoppers. The inoculum used for the batch bioreactor was the same as used for the flowthrough bioreactor and it is described therein.

Operation

Batch bioreactors were intended for one day runs; however, some experiments were run for several days. Their 100 mL head spaces were maintained with N_2 at 7 psig to assure

anoxic conditions. Bioassay bottles, including medium and metal coupon, were autoclaved at 15 psig for 15 min prior to each run or inoculation. Bacteria were inoculated at room temperature and placed in a Fisher low temperature incubator, model 307, at 37°C under aseptic conditions. One mL of E. aerogenes was inoculated first, then after 12 hrs one mL of each D. desulfuricans and C. acetobutylicum was added. Samples for pH and volatile fatty acids analysis were taken via the rubber stopper with a 5 mL syringe. Metal coupons were sampled aseptically and placed in 10 mL vials containing a cacodylate buffer, pH 7.2, to be further processed according to the protocol described in Table 3-1 for scanning electron microscopy examinations.

The GLYE media were prepared using ingredients outlined in Table 3-2, Table 3-3, and Table 3-4.

Electrochemical Cells Description

The new design of the batch bioreactor, here called the electrochemical cell, consisted of a modified 1-L erlenmeyer flask including an extra side opening that fit #8 butyl rubber stoppers. At the top opening port, an assembly of electrodes was fitted that included the working electrode (metal coupon), the pH combination Ag/AgCl reference electrode, polymer body unit/gel filled by Fisher Scientific, Co., and

pressure transducer by Setra Systems, Inc., model 205-2 and digital pressure indicator, model 300C.

The new system was instrumented for automated data acquisition of free corrosion potential, pH, oxidation-reduction potential, and head space pressure. Prior to filling the electrochemical cell to the 1-L mark with the medium electrolyte, the cell was outgased in an atmosphere of 80% N_2/20% CO_2. The flask was then sealed under a positive head space pressure and placed in a precision water bath by GCA corporation at 30°C. A head space pressure in the range of 20 to 40 mm of Hg above atmospheric pressure was used throughout the investigation. This was a pressure that allowed stoppers not to blow away, it was economical and allowed an adequate gas supply to the head space. A schematic of the electrochemical cell is detailed in Figure 3-3.

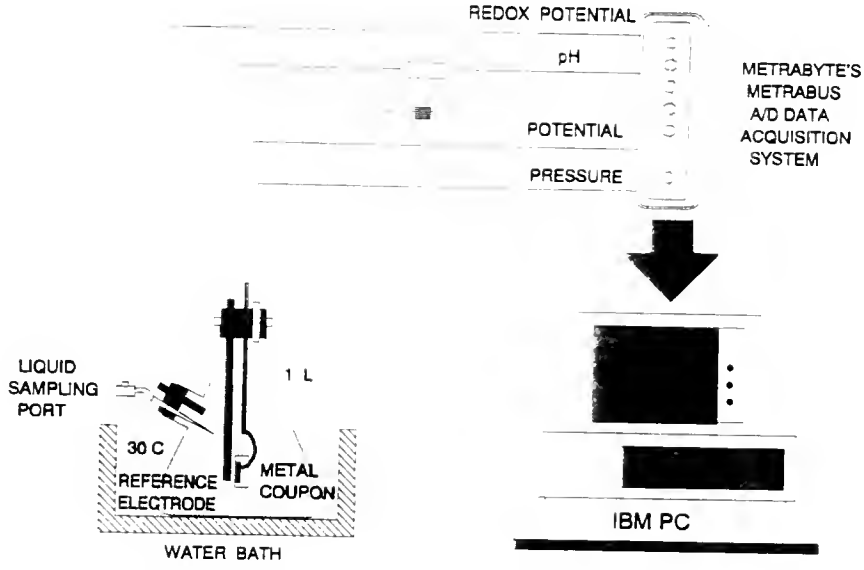


Figure 3-6. Schematic of electrochemical cell and data acquisition system.

The system set-up employed an IBM compatible computer with a four channel MetraByte's MetraBus A/D data acquisition system that interfaced the four parameters. Custom software, written in Quickbasic, was used to acquire pH and the three other parameters, free corrosion potential, oxidation-reduction potential, and pressure in the form of voltages. The liquid sampling port consisted of a Pasteur pipet connected to a 10 mL syringe via a clear plastic tube with an adjustable tube clamp.

Electrochemical Cells Operations

Single Flask Electrochemical Cell

The first modification or redesign of the batch bioreactor was the single flask electrochemical cell. A detailed schematic of the single cell is shown in Figure 3-4.

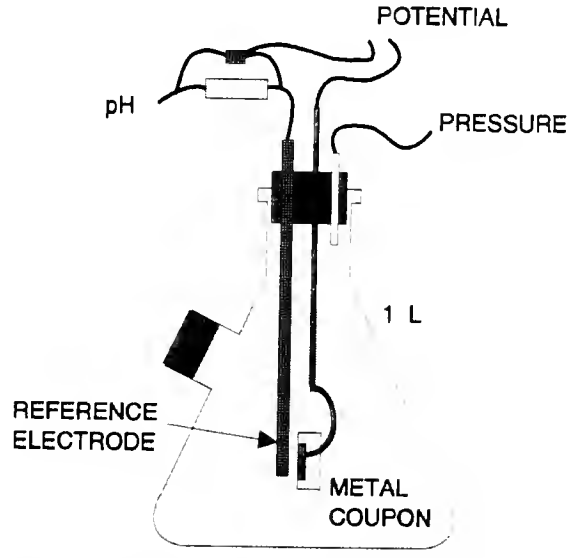


Figure 3-7. Single flask electrochemical cell.

Experiments using the electrochemical cells were run at constant temperature in a water bath at 30°C, described earlier. Anaerobic conditions were maintained throughout the experiments using gases treated in a 5.0 cm ϕ and 75 cm long copper column that was electrically heated to approximately 350°C and reduced with pure hydrogen. A gas mixture consisting of 80% N_2/20% CO_2 was used during outgasing, preparation of the mineral electrolyte, and preparation of the bacteria suspensions as described earlier in this chapter.

After outgasing the single cell, the electrochemical cell was filled with the mineral solution electrolyte to the 1 liter mark. Then, it was sealed with the stopper containing the metal coupon and electrode assembly, leaving a head space of approximately 200 mL at a pressure in the range of 20 to 40 mm of Hg above atmospheric pressure. At this stage, the metal coupon was held clear of the solution electrolyte and the complete cell was placed in the water bath. Resting cells harvested by centrifugation were supplied to the single cell by injecting them through the rubber stopper. Once the data acquisition system was connected to the SC and data started to be collected, the metal coupon was lowered into the electrolyte by pushing the insulated copper wire that holds the epoxy-mounted coupon through the rubber stopper.

Experiments using this electrochemical cell allowed fitting 4 single cells in the water bath for each run, one control and triplicate samples. Gas and liquid samples were

removed under sterile conditions, using appropriate syringes through the septums provided at the top and side flask openings.

Dual Flask Electrochemical Cell

It consisted of the single cell (flask I) interconnected to an additional flask II through their head spaces as described in Figure 3-5. The objective of flask II was to overcome some anticipated inconveniences experienced in the single cell such as metal toxicity and/or any stress imposed on bacteria during the harvesting procedure. Now flask II could allow bacteria to grow freely and out of contact with the metal to avoid metal toxicity. The head space connection allowed cathodically produced hydrogen to be transferred from flask I to flask II.

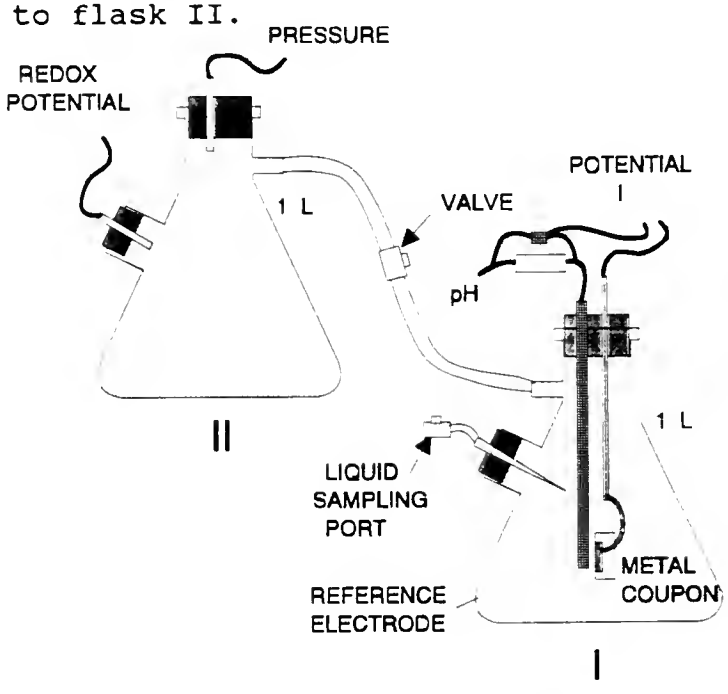


Figure 3-8. Dual flasks electrochemical cell.

Dual cell experimental runs followed a procedure similar to the setting and operation of the single cell and described above, except that flask II could be used to grow bacteria in their optimum media. The setting of the dual cell did not allow more than one dual cell in the water bath, for which only single experiments were run.

Triad Flask Electrochemical Cell

This final design consisted of a set-up similar to the dual cell including an additional flask III, which is interconnected to flask I via an ionic bridge made out of 5% agar, KCl saturated. Flask III was similar to flask II except the latter was connected to flask I by the head space. A detailed schematic is shown in Figure 3-6.

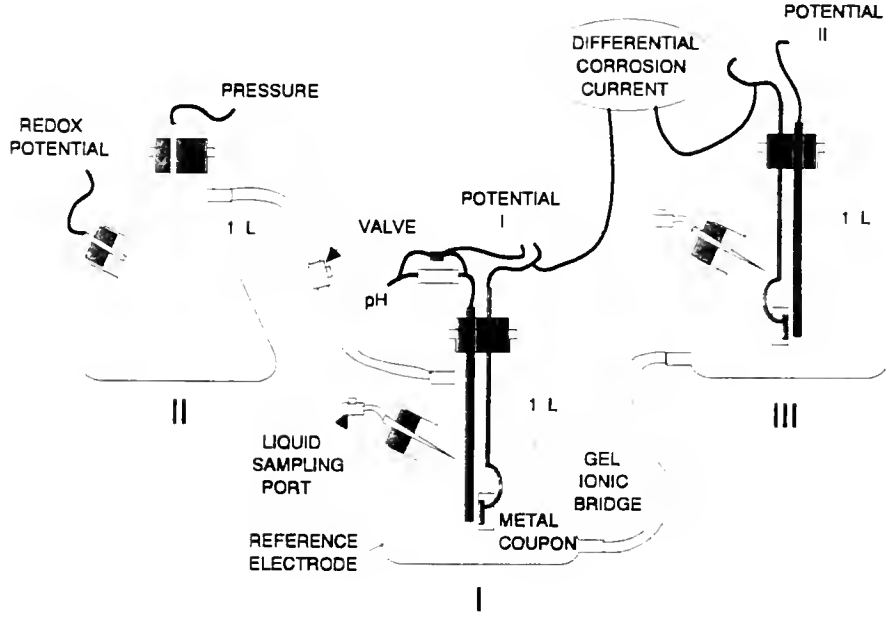


Figure 3-9. Triad flasks electrochemical cell.

This design was considered capable of satisfying the objectives of this research because it allowed the measurement of a differential corrosion current (DCC) induced by bacteria in addition to other parameters mentioned earlier in the single cell. A home-made zero resistance ammeter was incorporated in the data acquisition system in order to measure and acquire the new DCC parameter. The triad cell as well as the dual cell allowed bacteria to be grown in flask II or injected in as suspended cells.

The four available data acquisition system channels were used to acquire data on the following parameters: pH in flask I, free corrosion potential in flasks I and III, and DCC between metal coupons exposed and unexposed to bacteria in flasks I and III respectively. Triad cell runs used the selected metal AS106 and the selected bacteria Escherichia coli (JW111).

Liquid samples for dissolved iron determinations were taken simultaneously from flask I and flask III, every ten minutes. Only one triad cell fit the 30°C water bath.

Other experiments using the triad cell include a run in which a vacuum was applied to flask I and then to flask III in order to exaggerate a case of hydrogen uptake. Near the end of the test a positive hydrogen pressure was applied to flask III in order to observe its effect on the potential and differential corrosion current measurements. The last experiment consisted of a galvanic metal couple of Mg working

electrode in flask I and a Cu working electrode in flask III, to which vacuums were applied correspondingly to test the triad cell response to hydrogen uptakes in both flasks. The purpose of this test was to check the measurement capacity of the electrochemical system since a significant differential corrosion current was supposed to be developed.

Preparation of Bacterial Suspensions

The organisms used in this investigation were Desulfovibrio desulfuricans (ATCC 7757), Entrobacter aerogenes (ATCC 13048), Clostridium acetobutylicum (ATCC 824), Escherichia coli (ATCC 8739), Alcaligenes eutrophus (ATCC 29597), and Escherichia coli (JW 111), provided by Dr. K. T. Shanmugam. The first three bacteria have been found often in real MC cases and all of them represented species of hydrogen oxidizing bacteria.

The first three bacteria D. desulfuricans, E. aerogenes, and C. acetobutylicum were grown in glucose lactate yeast extract (GLYE) medium, as shown in Tables 3-2, Table 3-3, and Table 3-4. The rest of the bacteria were grown in Trypticase Soy Broth media. All chemicals used to prepare the media were reagent grade and were obtained from Fisher Scientific or Sigma Chemical Co. All media were prepared under an atmosphere of 80% N_2/20% CO_2. However, when microorganisms used for MC experiments were inoculated in their respective

medium, an atmosphere of 80% H_2/20% CO_2 was used to activate their hydrogen uptake enzymatic systems.

Stock cultures from ATCC were prepared at 30°C from freeze-dried ampules of the organisms in their recommended growth media indicated above. Cultures were maintained inoculating agar slants (monthly) and liquid media (weekly). 20 mL serum vials were used for facultative bacteria, and 25 mL crimp-top tubes, sealed with butyl rubber septa, for anaerobic bacteria.

Resting-cell suspensions were used regularly only in experimental set-ups that included electrochemical measurements; other experiments used bacteria inoculated directly from their grow media. The batch bioreactor also used washout from slants in some of its runs. Resting-cell suspensions avoid electrolyte contamination with media used to grow bacteria.

Cell suspensions were prepared as follows: 1 mL of subculture of facultative microorganisms less than 1 week old or 2 mL of subculture of anaerobic microorganisms were inoculated in 400 mL of appropriate growth media and incubated in a Fisher low temperature incubator, model 307, at 35°C under a 80% H_2/20% CO_2 headspace for 1 day for facultative bacteria and 3 days for anaerobic bacteria. Once cells were grown, they were harvested by centrifugation in 50 mL screw cap tubes for 10 min at 10,000 g.

Bacteria pellets from tubes were then resuspended in 5 mL of the mineral solution electrolyte to give an approximate concentration of the cell suspension of 65 mg (dry weight basis).

Preparation of Mineral Solution Electrolyte

In order to provide the electrochemical cell with minimum nutrient conditions as encountered inside gas transmission pipelines and still allow bacteria to survive for few days, a mineral solution electrolyte was developed to be used in the experimental system to study the onset of microbial corrosion. The ingredients of the mineral solution electrolyte consisted of 10% of the concentration of the components of solutions specified in Table 3-4 including 1 mg/L of resazurine and deionized distilled water to complete the required volume concentration. The ingredients were heated to the maximum mark on the dial on a Fisher Scientific hot plate, model 210T and stirred under an 80% N_2/20% CO_2 atmosphere. After heating for 0.5 hr, the flask was cooled down to 30°C surrounded by crushed ice in a plastic container for approximately 1 hr. At this time, the electrolyte was transferred to previously cleaned electrochemical cells and filled to the 1-L marks. Remaining electrolyte was used to resuspend bacteria and other routine tests.

Metal Coupon

From an original collection of 33 different metals and alloys, only 18 different metal samples were cut and coupons fabricated. Samples were prepared based on availability and ease of cutting to the maximum size allowed in the molds used for epoxy mounting. Metal coupons used in the flowthrough and batch bioreactors were not epoxy mounted.

The availability of SA106 steel coupons, its non-toxic effects on bacteria, its successful surface colonization and observed surface deterioration, and the popular use of the alloy in gas production pipelines suggested its selection for the electrochemical experiments. In addition, it has a moderate hydrogen production and it is relatively easy to polish.

A technique was developed to fabricate the working electrodes used in the electrochemical cells. It consisted in cutting metal coupons from ASTM-ASME SA106 grade B1 steel, 2.5 cm diameter schedule 80 17-24 H.T. 2500 psi pipe, obtained from Texas Eastern, Louisiana. The metal coupons were machine ground to a size of 1 cm x 1.9 cm x 0.02 cm, and attached to 30 cm long 12 THWN insulated hard copper wire by Cerrowire using nickel print paint (cat. No. 22-207) by GC Electronics. Coupons were centrally mounted on epoxide resin 20-8130-128 and hardener 20-8132-032 from Buehler, leaving an area of 1.0 cm x 1.9 cm exposed for the microbial corrosion studies. The

hard wire served to support and to electrically connect the coupon and was thereby insulated from contact with the electrolyte in which the working electrode was immersed. Prior to the start, the working electrode was held clear of the solution contained in the flask and lowered into the solution at will by pushing the hard copper wire through the butyl rubber stopper that holds the electrode assembly.

After preparation, the working electrodes were polished with 600 grit, rinsed with ethanol and blotted dried with Kimwipes EX-L delicate task wipers, then stored in either a desiccator glass container or a desiccator cabinet by Labconco Co., model 55300, under vacuum until ready to be used. During the polishing of the working electrodes, they were initially sanded with a 240 emery grid paper to remove any inconsistencies on the metal surface, then with a 400 grid, and finally with a 600 grid. The working electrodes were placed in an ethanol bath in the ultrasonic cleaner to ensure the removal of metal particles left from previous polishing. The polishing procedure was done using a Buehler rotary polishing wheel and care was taken so that no cross-hatching was left on the metal surface.

Parameters Setting

In an effort to assure consistency during the electrochemical measurements, several parameters were fixed

based on equipment capabilities, typical inside conditions of gas transmission pipelines, and other anaerobic stagnant conditions to make measurements simpler and more reliable. This task involved the use of the batch bioreactor and consisted of the study of several determining factors that formed the basis for the development of the electrochemical system described herein. Experiments done included determination of head space gas composition, selection of reducing agent, selection of sterilization method, and other related studies.

The head space gases used were methane, hydrogen, nitrogen, carbon dioxide, and helium. The reducing agents tested were sodium sulfide, cysteine, thyoglycolate/ascorbate, and Enterobacter aerogenes as a natural reducing agent. Among the sterilization methods used were: 1) all components of batch bioreactor autoclaved at 15 psig during 15 min, 2) all components of batch bioreactor autoclaved except metal-coupon-rubber-stopper assembly that was oven treated at 105°C for 12 hrs, 3) media autoclaved as before and metal-coupon-rubber-stopper assembly sterilized in acetone and ethyl alcohol for 15 min then blotted dry prior the set-up in bioassay bottle, and 4) media components autoclaved and metal coupon flamed. The basic anaerobic and stagnant conditions included fixed temperature of 35°C and pH in the range of 6.0 and 6.5.

Measurement Procedures

All biocorrosion experiments, using electrochemical cells, were performed at 30°C. Flasks using electrode assemblies include a rectangular metal coupon (working electrode) with surface areas of 1.9 cm^2 mounted in epoxy resin and abraded to a 600 grit finish. Metal coupons were connected to 30 cm of insulated hard copper wire that served as electrical conductors and support in the stopper. Electrode assemblies included Ag/AgCl reference electrodes, polymer body unit/gel filled, from Fisher Scientific. Reference electrodes were continuously calibrated against each other using the data acquisition system prior to the experimental runs. The side opening of flasks contained platinum electrodes for oxidation-reduction potential measurement and Pasteur pipets attached to a 10 mL syringe for liquid sampling.

The four data acquisition channels were connected to the triad cell as follows: channel four was wired to a pH reference electrode for pH readings. Channel one and channel two were wired to the working electrode and reference electrode of flask I for free corrosion potential measurements. Channel two was wired to the working electrode and reference electrode of flask III for free corrosion potential measurements, and channel three was wired to the working electrodes of flasks I and III for differential corrosion current measurements. Current was measured with a

homemade zero resistance ammeter with a 10 nA sensitivity, made of a #308 operational amplifier and a high precision 10,000 Ω resistor. This device has the property of converting current into voltage out with a gain determined by the high precision resistor, allowing the data acquisition system to measure and acquire data in volts. A digital pressure indicator, model 300C by Setra Systems was also adapted to monitor the headspace pressure in flask I. Once the data acquisition system was started the working electrodes were immersed in their respective electrolytes and data was stored in computer floppy diskettes.

Samples of bacteria, in their growth medium, in their resuspended medium, and in the liquid discarded after centrifugation, were homogenized using a vortex Fisher brand, model Genie 2, and taken to a spectrophotometer Spectronic 21D by Milton Roy for determination of transmittance, absorbance, and concentration at the start of each experiment. After spectrophotometer determinations, samples were vacuum filtered using 47 μm pore size membrane filters, preweighted in a Metler AE100 balance. Wet mounts were also prepared regularly to check bacteria viability under a light microscope by Nikon, model LABOPHOT-2. At intervals of 10 minutes, 10 mLs aliquots were withdrawn from the electrolytes from the flasks I and III under stagnant conditions and analyzed for dissolved Fe at the IFAS Soil Science Analytical Research Laboratory. Last runs were sampled only at the beginning and at the end, shaking

flasks to avoid stagnant conditions for dissolved iron. The duration of each electrochemical experiment was approximately 1 hour. Experiments were continued until the corrosion potentials leveled off.

Analytical Procedures

Dissolved iron analyses were performed at the IFAS Soil Science Analytical Research Laboratory. Samples were collected in 10 mL vials and preserved using 1 drop of concentrated H_2SO_4 and analyzed on an atomic absorption unit by Perkin Elmer.

Molecular hydrogen was measured on a gas chromatograph by Gow-Mac Instruments Co., series 580, equipped with a thermal conductivity detector. 50 μL samples were collected in a gas tight syringe and injected onto a molecular sieve type 5A column. Samples were injected at an inlet temperature of 40°C with column temperature of 35°C and a detector temperature of 93°C. The carrier gas used was nitrogen and the detector current was 50 mA.

Transmittance, absorbance and concentration were measured on a spectrophotometer Spectronic 21D by Milton Ray. The samples were collected in 10 mL Hatch COD tubes and homogenized in a vortex mixer by Fisher, model Gene 2, prior to measurements.

Volatile fatty acids were measured on a FID gas chromatograph by Shimadzu, model GC-9AM. The samples were

prepared by centrifugation after acidification with 20% phosphoric acid. The samples were injected onto a 2m long by 2mm ϕ glass column packed with 80/100 chromosorb 1200 WAW coated with 3% H_3PO_4 and carried with nitrogen gas. 1μL volumes were injected at an inlet temperature of 180°C with column temperature ramped from 130°C to 170°C over 5 min and a detector temperature of 200°C.

Dry weights of resting cells were determined by vacuum filtering known volumes of bacterial suspensions. Filtration was performed in a 47 mm magnetic Gelman Filter funnel (cat. 09-735). The samples were filtered using 0.45 μm pore size membrane filters, 47 mm plain Supor 450, by Gelman Sciences, Co.. Vacuum was achieved with a precision belt-driven vacuum pump by Fisher Scientific, model D-75. Membrane filters were then oven dried at 105°C for 12 hrs. Weights were measured in a Metler balance, model AE100.

Gases

The gases used during the investigation were: Nitrogen (UN1066), Helium (UN1046), Hydrogen (UN1049), Nitrogen/Carbon Dioxide (80/20, UN1956), and Hydrogen/Carbon Dioxide (80/20, UN1954) supplied by Liquid Air Corporation. For anaerobic experiments all gases were passed through a heated copper column (5 cm ϕ and 75 cm long) to free gases from traces of oxygen. The column was heated electrically to about 350°C by a coil of electrical wire wrapped around the column.

Reduction of the column was achieved by passing hydrogen gas through the column.

Scanning Electron Microscopy

Metal coupons, after exposure to microorganisms, were treated with a buffered glutaraldehyde solution to fix the morphology of the bacteria, and then dehydrated through a graded ethanol series of increasing concentration ranged from 25% to 100% using procedure outline in Table 3-1, page 93. Samples were then dried and sputter coated with gold palladium, then examined in a Hitachi S-450 SEM, that included an Energy Dispersive X-ray Analyzer (EDXA). Coupons were analyzed throughout and micrographs and spectra were taken at sites of major interest.

Light Microscopy

Metal coupons were observed and photographed under a Nikon stereomicroscope, model SMZ-2T and a Nikon microscope, model LABOPHOT-2, for biofilm and corrosion examination. Pure cultures and liquid samples from bioreactors were examined periodically for contamination via wet mounts.

CHAPTER 4
RESULTS AND DISCUSSION

Flowthrough Bioreactor

Two experimental runs were performed in this bioreactor in order to reproduce microbial corrosion and to study the effect of a bacterial triculture on the corrosion of carbon steel coupons and the formation of biofilm. Its set-up is described in Figure 3-1. Both runs reproduced microbial corrosion and their results are shown in Figures 4-1, 4-2, 4-3, 4-5, and 4-6. Metal coupons exposed to a bacterial triculture, grown in glucose lactate yeast extract (GLYE) media under anaerobic conditions, were examined under the light and the scanning electron microscope (SEM). The results indicated extensive bacteria colonization as well as metal deterioration. Careful observations of biofilm micrographs have suggested a mechanism for understanding its formation.

The first experiment was run for a period of four weeks. Metal coupons and liquid media were sampled weekly for routine analyses. First week scanning electron microscopy (SEM) micrograph results indicated that the bacterial triculture developed a biofilm consisting of a separated polymeric double

layer structure; an inner structure attached to the metal surface, and an outer structure that covers it. It appears that Enterobacter aerogenes, the bacterium inoculated first, was responsible for the production of the inner structure and perhaps the outer structure of the biofilm, see Figures 4-1a and 4-1b. This bacterium, besides reducing the media to accommodate the strict anaerobes, synthesized a considerable amount of extracellular polymeric material for attachment purposes. This material is believed to overcome the natural surface repulsion caused by the negatively charged bacteria and metal surfaces (Beveridge and Doyle, 1989). Figure 4-1c shows the characteristics of the abundant polymeric network-like inner structure produced by the bacterial triculture. E. aerogenes is probably responsible for building this structure because it is the most prolific of the three species and reaches the log phase of the growth curve in less than 6 hrs as compared to the other two bacteria which require often more than two days. Wachenheim and Patterson (1992) reported that anaerobic production of extracellular polysacharides is enhanced generally with any improvement of the conditions of bacterial growth and that this material is only produced during the log phase of growth where its production is also exponential. In our work, the flowthrough bioreactor was run using GLYE medium which must have enhanced the production of the polymeric material. This fact, if compared to experiments run in a medium with limited nutrient composition (mineral

solution only), indicates that the amount of biofilm formed on the metal surface is proportional to the amount of organic nutrient in the medium.

Desulfovibrio desulfuricans and Clostridium acetobutylicum were inoculated one day after the initial inoculation of Enterobacter aerogenes. The components of the bacteria triculture were cultured separately. Figure 4-2 shows scanning electron microscopy micrographs of each species for identification purposes. After inoculation, the strict anaerobes probably displaced E. aerogenes from the inner structure of the biofilm so it moved upward and started producing the outer biofilm layer. This mechanism, suggested in Figure 4-1a, indicates the presence of D. desulfuricans and C. acetobutylicum in between the inner and outer structure and Figure 4-1c shows that most bacteria in the outside are represented by E. aerogenes and C. acetobutylicum. These figures also show that the outer biofilm structure is rougher on the inside than on the outside. The inner surface seems like a woven net of polymeric material attached to the metal surface.

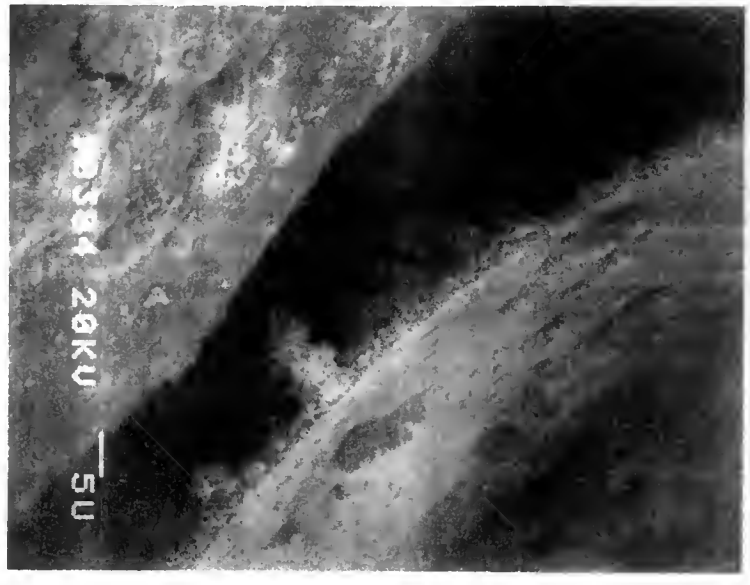
Metal coupon surfaces for this experiment were polished to 240 grid. Figure 4-3a represents a micrograph of the control surface, unexposed to bacteria and magnified 1200 times. Figure 4-4a shows the corresponding energy dispersive x-ray analysis (EDXA) spectrum of the metal surface.

After anaerobic exposure of the metal coupons to the bacterial triculture for a week, surface colonization including pitting corrosion was observed. Figures 4-3b, 4-3c, 4-3d, and 4-3e show localized pitting and polymeric biofilm,

a)

Figure 4-1. Flowthrough bioreactor results on bacteria attachment to carbon steel coupons. a) SEM micrograph showing bacteria within the outer and the inner structures of the biofilm. b) SEM micrograph indicating the nature of the outer biofilm structure.

b)



c)

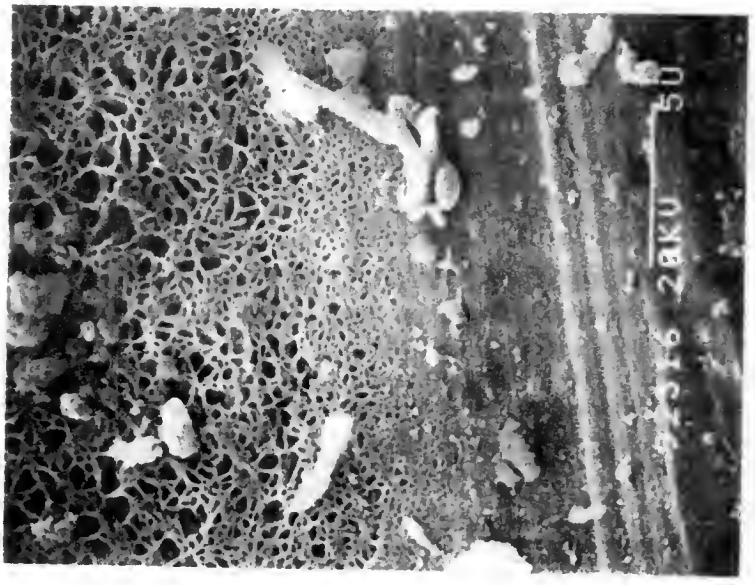


Figure 4-1. c) SEM micrograph showing bacteria on inner biofilm.

Figure 4-2. SEM characterization of bacterial species from triculture. a) Enterobacter aerogenes.

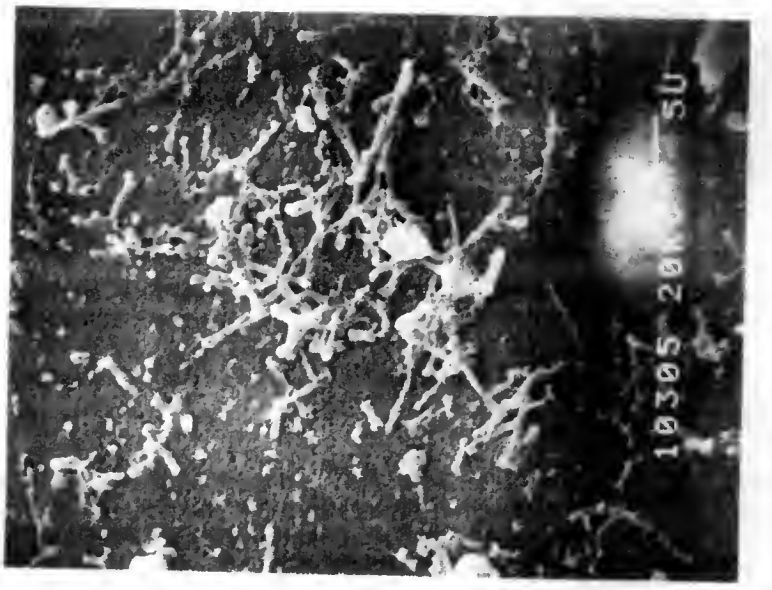


Figure 4-2. b) Clostridium acetobutylicum.

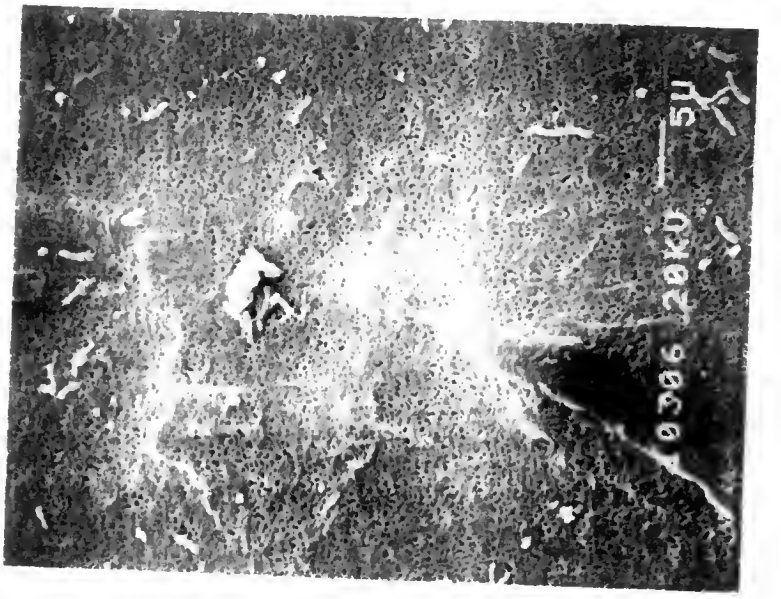


Figure 4-2. c) Desulfovibrio desulfuricans.

metal deterioration near the edge of the biofilm, generalized metal deterioration, and elongated pits respectively. Figure 4-4b shows the EDXA spectrum of a metal coupon after exposure to the bacterial triculture. This analysis presents additional evidence of microbial corrosion. It shows the presence of an additional peak (if compared to the control), corresponding to a sulfur compound, which is probably due to the deposition of ferrous sulfide resulting from the activity of D. desulfuricans.

Stereomicroscopic observation of coupon surfaces after exposure to the bacterial triculture suggest the nature of the biofilm to be slimy. Figure 4-5a and 4-5b are photographs of the face and edge of a metal coupon respectively.

During the initial run of the flowthrough bioreactor, volatile fatty acids and pH were measured weekly for one month. Results, shown in Figure 4-6, indicate that the only VFAs produced were acetic and butyric acids. During the first week, only acetic acid was produced and its concentration ranged between 300 and 400 ppm until the 3rd week and almost double by the end of the fourth week. Butyric acid appeared after the second week and increased at a rate of 200 ppm per week to a concentration very close to 600 ppm at the end of the 4th week. Figure 4-6 also shows that pH dropped to 6.5, probably due to the accumulation of volatile fatty acids. During the first three weeks the pH ranged between 7.0 and 7.5 then dropped to 6.5 during the 4th week.

Figure 4-3. Flowthrough bioreactor SEM results of microbial corrosion of carbon steel coupons. a) Control surface of coupon unexposed to bacteria and polished to 240 grid.

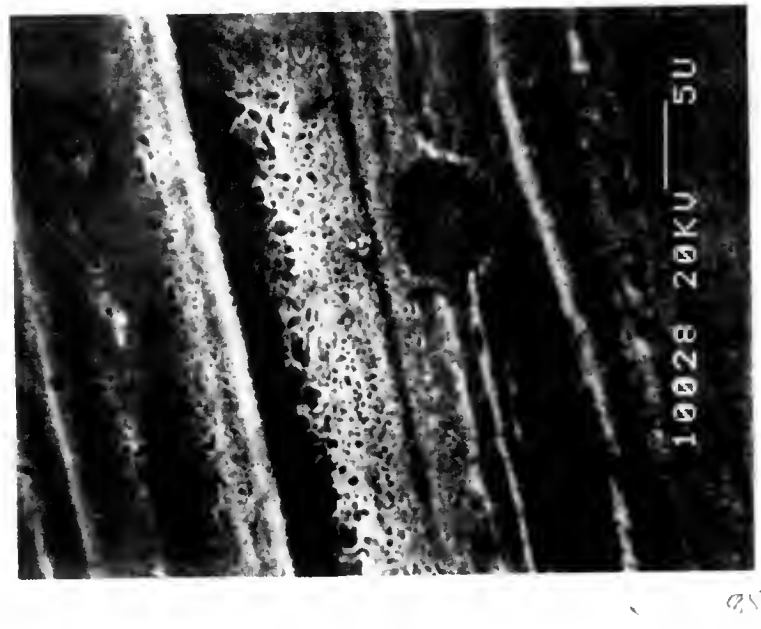


Figure 4-3. b) Localized pitting near biofilm after exposure to bacterial triculture.

Figure 4-3. c) SEM micrograph showing microbial corrosion near biofilm.

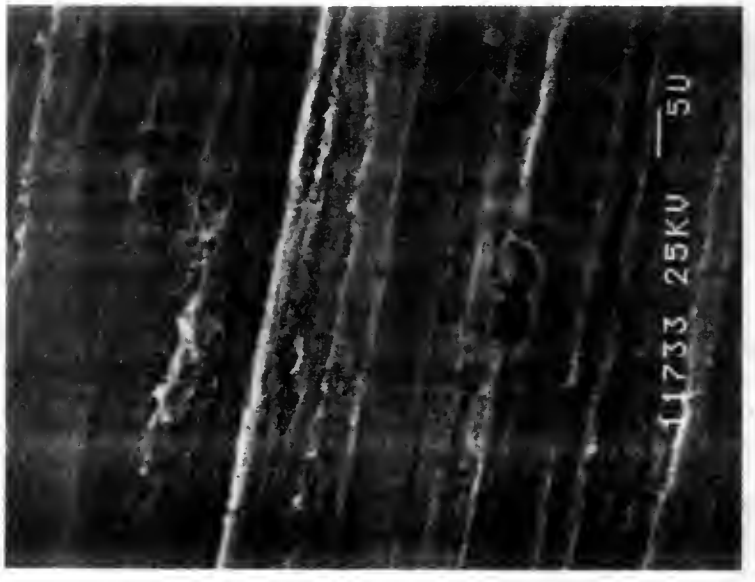


Figure 4-3. d) SEM micrograph showing generalized metal deterioration after exposure to bacteria.

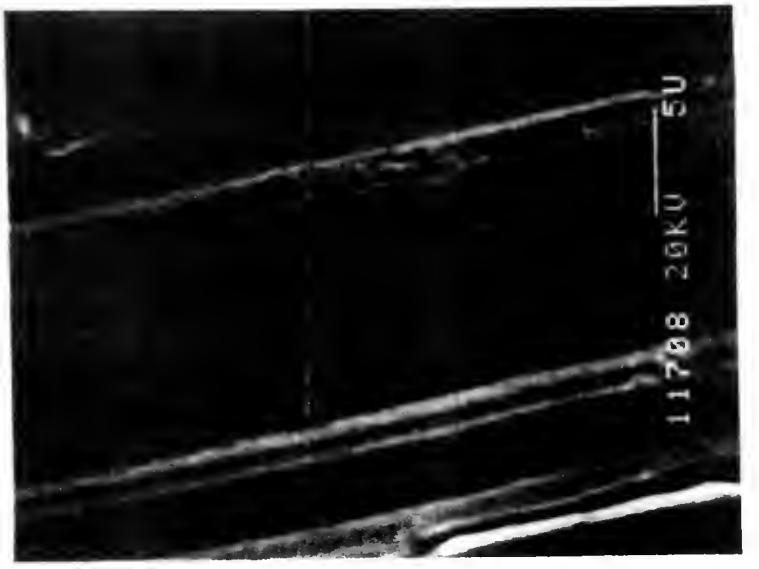


Figure 4-3. e) SEM micrograph showing elongated pits.

Figure 4-4. Flowthrough bioreactor results of EDXA analysis. a) Control EDXA spectrum of a steel coupon unexposed to bacteria indicating metallic components of the SA106 alloy.

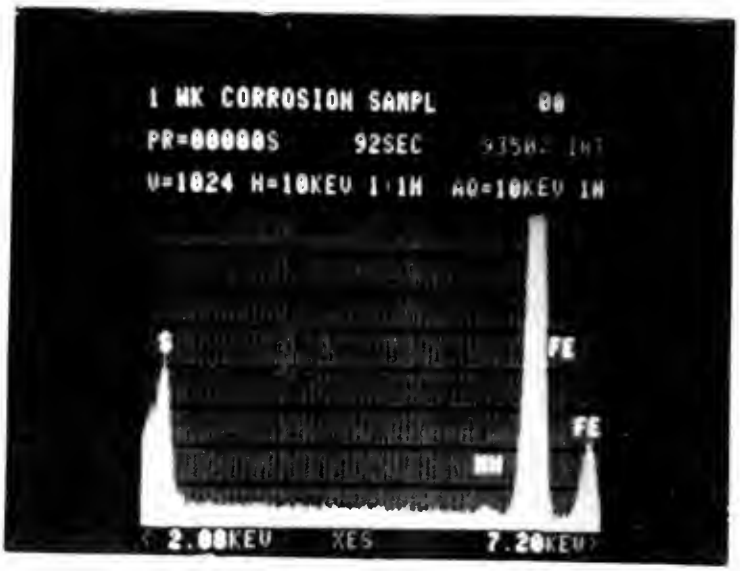


Figure 4-4. b) EDXA spectrum of a metal surface exposed to bacteria showing the sulfur peak resulting from metal sulfides deposition by D. desulfuricans..

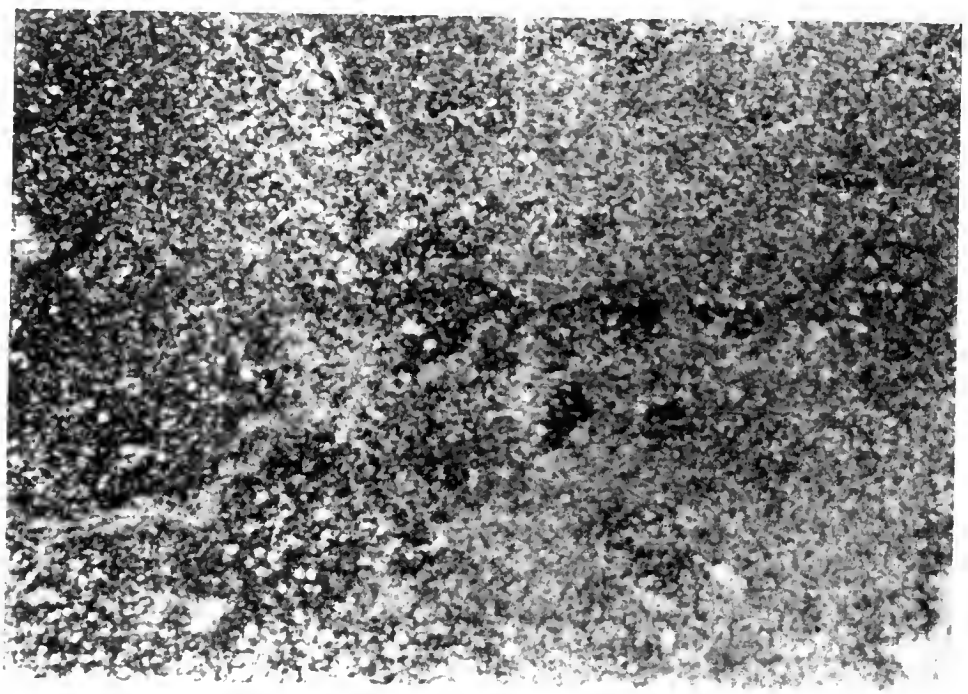


Figure 4-5. Flowthrough bioreactor biofilm appearance under stereo microscope. a) Flat-side view.

Figure 4-5. b) Biofilm edge-side view.

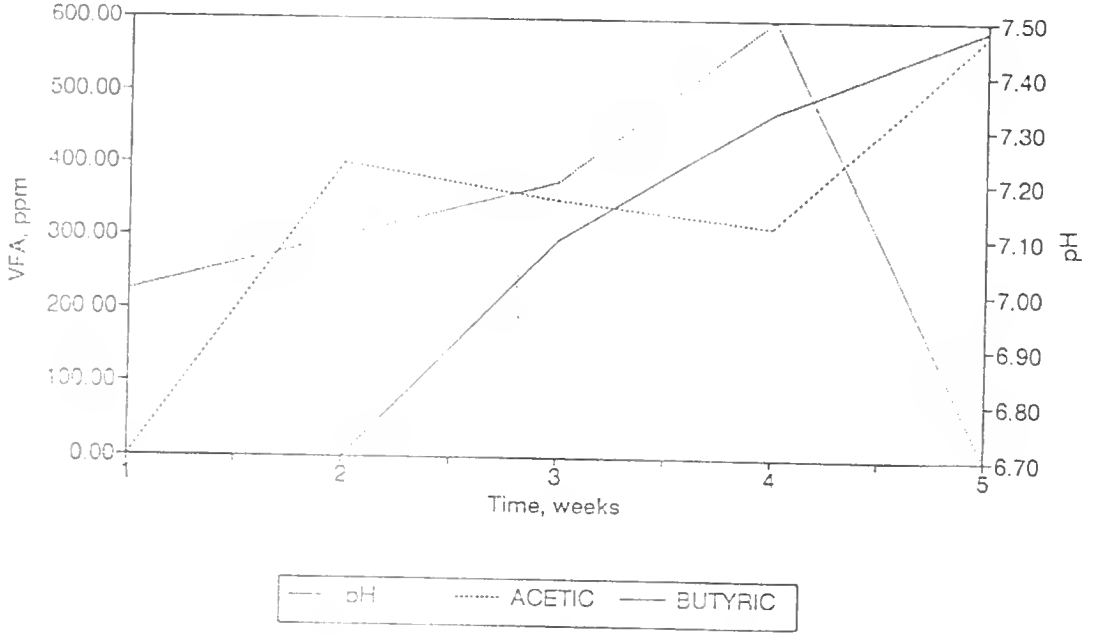


Figure 4-6. Flowthrough bioreactor volatile fatty acids and pH profile.

A second experiment with the flowthrough bioreactor suggested that biocorrosion initiates within 24 hours. Figures 4-7 and 4-8 show SEM micrograph and EDXA spectrum of a metal coupon sampled one day after the start of the run. Results of this experiment were similar to the previous one. Figure 4-7a shows the presence of the extracellular polymeric material attached to the metal surface and Figure 4-7b shows metal deterioration, including pitting corrosion comparable to previous results. Figure 4-8 indicates a similar peak corresponding to sulfur compounds which may be attributed to deposition of metal sulfides by D. desulfuricans. In general, the results of the second experiment showed that the effect of the bacterial triculture on the corrosion of carbon steel produced similar types of biofilm and pitting but to a lesser degree.

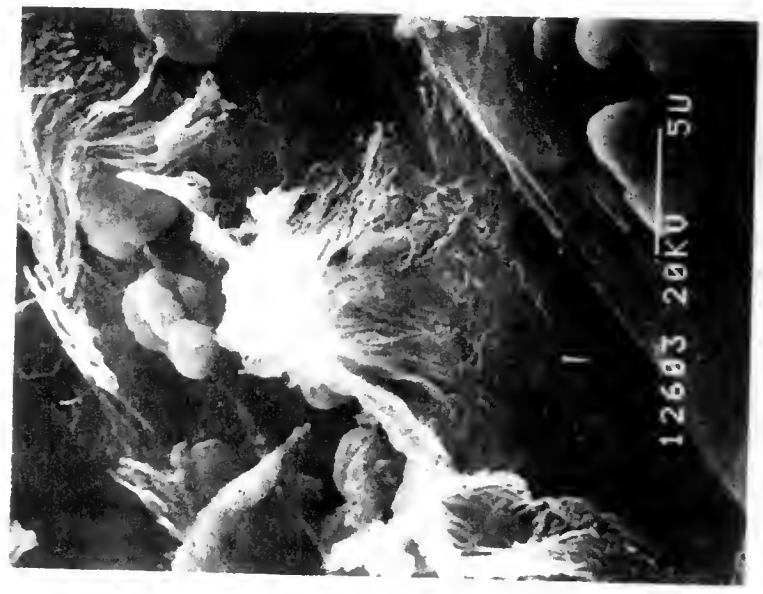


Figure 4-7. Flowthrough bioreactor-second experiment. a) SEM micrograph showing appearance of biofilm.

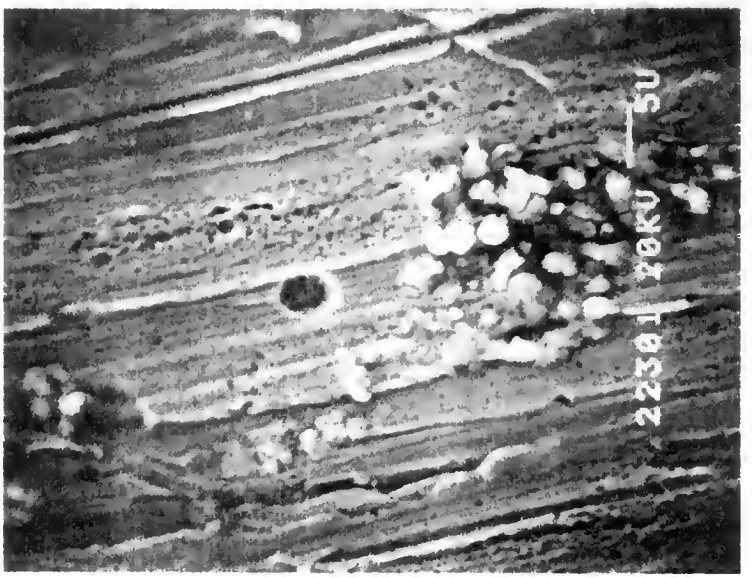


Figure 4-7. b) SEM micrograph indicating massive pitting corrosion.

Figure 4-8. Flowthrough bioreactor EDXA spectrum of metal coupon exposed to bacteria showing the sulfide peak.

Volatile fatty acids analysis indicated levels of 140 ppm for acetate after one day and reached a value of 340 after one week while pH remained constant in the range of 7.5 and 7.2.

Batch Bioreactor

Based on the results of the flowthrough bioreactor, the theory of cathodic depolarization, and the literature reviewed on organic acids'effect on corrosion (Soraco et al., 1988; Little and Wagner, 1987; and Beveridge et al., 1983), a new batch bioreactor system based on the principles of the bioassay bottle experiment was used. The rationale here was to study the initial steps of the microbial corrosion process that could set the stage for the development of an experimental system that would be able to quantify the biological component of corrosion, in addition of using a simpler bioreactor. It was necessary in this system to avoid the formation of a biofilm allowing bacteria to live for at least six hours, and then detect the effect of their activity on the metal surface.

Using the batch bioreactor set-up described in chapter 3, a series of experiments were performed to reproduce microbial corrosion at conditions resembling the inside of gas transmission steel pipelines. The research included a

preliminary study of carbon and energy sources required by the bacterial triculture to survive one day.

Results show that the batch bioreactor reproduced microbial corrosion within 24 hours. The system allows for visual inspection of exposure of metal coupons to living bacteria and to check for changes of parameters such as head space gas and media composition. It is less prone to bacterial contamination and medium does not need to be replenished as in the flowthrough bioreactor. These characteristics showed the batch bioreactor to be superior to the flowthrough bioreactor, in addition to being more attractive economically.

Effect of Head Space Gas Composition

Head space gases used were H_2, CH_4, 50% H_2 / 50% CH_4, N_2, 80% N_2 / 20% CO_2, 80% CH_4 / 20% CO_2, and He at a pressure of 7 psig. Head space gases were used in combination with carbon steel coupons and variations of GLYE media compositions, the bacterial triculture, and tar-condensate product from an offshore gas production platform.

Results show that the head space affected the bacteria/metal/fluid components. Results using GLYE media indicate that for all head space except the controls, the bacteria triculture caused the formation of a black precipitate that adhered to the steel surface and also remained suspended in the liquid. Bottles with methane in the

head space produced an additional shiny, black, uniform film at the liquid interface. In the case of hydrogen, the interfacial black precipitate was not formed. Results using a GLYE media containing 10% of the original concentration of glucose and lactate, show similar results to the flowthrough bioreactor experiment except that a thicker black film was formed on coupons under the nitrogen head space. Results using GLYE media, excluding glucose and lactate, also produced a black precipitate on the metal surface and in the bulk of the fluid.

Coupons in bottles with nitrogen/carbon dioxide and methane head space were uniformly covered with the black film. Results using the mineral components of the GLYE media produced similar results to the previous experiment.

When the medium was composed of only yeast extract and vitamins, no black precipitate was formed either on the metal surface or in the fluid. Results of the control experiments using distilled water for the medium showed no black precipitate either. Other control experiments using the GLYE media composition combinations mentioned above, excluding only yeast extract and vitamins in the absence of bacteria, produced a slight darkening of the fluid after a week and the production of a crystalline film on the surface of the metal coupons.

Effect of Bacterial Combination

Based on previous results and the ability of sulphate reducing bacteria to reduce sulphate to sulphide, an experiment was set-up to determine the bacterial combination responsible of the black precipitate. Of the seven bacteria combinations tested, only the ones containing D. desulfuricans produced the black precipitate in both the metal and the fluid. In order to reduce the chances of production of the black precipitate, washings of bacteria cultured on agar slants were used to minimize bacterial metabolites and other undesirable substances contained in the culture medium during inoculation.

Selection of Media

Some complementary experiments to the head space experiment were performed in order to select the medium that will resemble inside conditions of gas transmissions pipelines and allow the study of cathodic hydrogen utilization at the onset of the microbial corrosion process. The medium composition included combinations of the following components; yeast extract and vitamins, minerals, glucose and lactate, and minerals excluding phosphates and/or sulphates. A nitrogen head space and a bacteria combination, D. desulfuricans and E. aerogenes, were used for all combinations of the media components. Results indicate that medium excluding phosphates and sulphates did not allow the black precipitate to occur in

either the liquid or the on metal surface. The amount of organic nutrient was directly related to the amount of black film deposited on the metal coupons. Consequently, bottles with GLYE medium produced the darkest fluid and the most biofilm on the coupons. Results with 10% strength mineral components of the GLYE medium, less organic components produced the most acceptable results. It produced the least turbidity and the least amount of biofilm. Further tests with the 10% strength mineral solution showed that it could maintain bacteria alive for over 6 hours, its normal pH is 6.5 but can be easily lowered to 4.5 with CO_2. Based on the above facts and other reasons to be discussed, the 10% strength mineral solution was selected as the electrolyte solution to be used in all the electrochemical experiments.

Selection of a Reducing Agent

Early in the use of the batch bioreactor systems, it was necessary to select a reducing agent for the media that would assure the low redox conditions required by the strict anaerobes but not react or interfere with the components of the microbial corrosion system. Several reducing agents normally used in anaerobic studies including a natural bacterial reducing agent were tested to study their effects on the metal and on the liquid media. Three chemical reducing agents tested included sodium sulphide, cysteine, thyoglycolate/ascorbate, and a selected facultative anaerobic

bacterium, E. aerogenes, were tested in the mineral electrolyte solution.

Results indicate that all chemical reducing agents were able to reduce the medium at their recommended low concentrations, but they produced a black film on the metal coupon upon contact with the liquid media. The bacterial reducing agent was also able to reduce the media, but it was concentration dependent. Five mL of bacteria per 150 ml of mineral solution were able to reduce the media in 6 hours. However, 3 mL / 150 mL reduced the media in two days. The bacterial reducing agent developed some turbidity after a week.

Selection of a Sterilization Method

The selection of a sterilization method was another task that required attention at the beginning of the tests with the batch bioreactor. The most practical method was autoclaving of the coupon and the medium together in a final set-up, ready for inoculation. This method was inappropriate because it allowed the steel coupon to rust, and rust accumulated at the bottom of the bottle. For this reason two additional methods were tested. They were: a) autoclaving medium and treating the stopper holding the metal coupon in an oven at 105°C for 12 hours and b) autoclaving media and treating the stopper holding the metal coupon in an acetone-alcohol bath for 15

minutes then blotting it dry. Autoclaving in all cases was done at 15 psig and 15 minutes.

Results indicated that the most convenient method that assures less bacterial contamination and prevents the metal from corroding was autoclaving the medium and separately sterilizing the metal coupon in an oven. The sterilized coupon was then aseptically suspended in the medium. This method was selected and after several trials, as the metal coupons remained clean and shiny inside a clear mineral electrolyte solution for over 3 days.

Experimental Results

The batch bioreactor experiments were analyzed for volatile fatty acids, SEM, EDXA, head space gas composition and pressure. Table 4-1 shows the results for volatile fatty acids from experiments using the mineral solution, metal coupon, bacterial triculture and head spaces containing N_2 / CO_2, H_2, CH_4, and CH_4 / H_2. Volatile fatty acids results were expected to be zero. However, values in the range of 9 to 195 ppm were obtained from these experiments after two days. Detectable values might be attributed to volatile fatty acids already present in the inocula as they were taken from their normal cultures that used media rich in nutrients and/or to gas chromatograph calibration sensitivity.

Table 4-1. Batch bioreactor volatile fatty acids measurements from experiments using steel coupons and bacterial triculture in mineral solution.

	Volatile fatty acids, ppm			
Gas head space	Acetate	propionate	isobutyrate	butyrate
N_2/CO_2	190	43	93	--
N_2	81	--	--	--
CH_4	112	114	--	--
H_2	81	45	--	--
CH_4/H^2	126	46	--	23

Figure 4-9 shows the structure of the biofilm formed in the GLYE medium within 24 hours. Bacterial counts per area (mm^2) of metal surface in a 4 hour sample, indicated concentrations of 15,000 bacteria cells. Figure 4-10 shows a) bacteria colonization within 24 hours and, b) its corresponding EDXA spectrum indicating the sulfur peak characteristic of the activity of D. desulfuricans.

Figure 4-9. Batch bioreactor biofilm formed within 24 hours.

Figure 4-10. Batch bioreactor SEM and EDXA results on bacteria attachment to carbon steel. a) Bacteria colonization within 24 hours.

Figure 4-10. b) EDXA spectrum of metal coupon exposed to bacteria for 24 hours.

Head Space Gas and Pressure.

Head space gas composition and pressure were analyzed and measured periodically to check anaerobic conditions and detect gas leaks. Gas analysis aided in the explanation of observed abnormal surface conditions resulting from air leaks. During the experiments with the batch bioreactor, it was observed that in 2% of the runs the head space became contaminated with air. All cases had bad stoppers and as a result coupons rusted from the start of the runs. Gas analysis in all cases showed an oxygen concentration in the range of 6 to 9%.

Results in Table 4-2 indicated that head space gases show pressure drops within one day and then stabilize thereafter.

Those results suggested the utilization of head space gases by bacteria in their metabolic processes to different degrees during their contact with the metal coupon. The head space gas utilization was greater in experiments using mineral solution than in experiments using GLYE medium. It was interesting to notice that, in all bottles containing nitrogen, the pressure drop was 100%. However, in the case of the GLYE media, the pressure drop for hydrogen was 48.0% and for methane 47.8%. In the case of the mineral solution, the hydrogen pressure drop was 72.0% and the methane pressure drop was 56.0%. Control experiments containing hydrogen in their head space and no bacteria had a pressure drop of 36.0%.

Table 4-2. Batch bioreactor head-space pressure drop in GLYE and mineral solution media after two days.

	Pressure drop, %	
Head Space Gas	GLYE	Mineral Solution
N_2 / CO_2	100.0	100.0
N_2	100.0	--
H_2	48.0	72.0
CH_4	47.8	56.0
Control	36.0	36.0

These results suggested that bacteria in the nutrient limited medium metabolized more head-space gas than in the medium rich in nutrients which might constitute a source of more readily available energy source. These results were further confirmed

in other experiments performed to study hydrogen uptake by hydrogen oxidizing bacteria. Results of those experiments indicated larger hydrogen uptake when the medium lacked organic nutrients.

Crystalline Films.

Figure 4-11a shows the SEM micrograph of the structure of a crystalline film that formed after one week in the absence of bacteria. Figure 4-11b represents the corresponding EDXA spectrum showing the peak corresponding to phosphorus. The spectrum suggested the presence of a phosphorous compound in the crystalline film that formed on the metal surface. Crystalline films were observed on metal surfaces approximately one week after exposure to the mineral solution in the absence of bacteria, or after one week bacteria had died. Figure 4-12a shows a transition from the biofilm to the crystalline film where the crystalline film formed several days after all the bacteria had died and decayed. Figure 4-12b shows a pitting corrosion and deterioration of the polymeric network like biofilm material after a gentle removal of the crystalline film with a nylon brush.

The results of the anaerobic batch bioreactor system were used as the basis for the development of an experimental system in which an attempt was made to quantify the biological component of corrosion during bacterial utilization of cathodic hydrogen.

Figure 4-11. Batch bioreactor - formation of a crystalline film on the metal surface after one week of exposure to the mineral solution in the absence of bacteria. a) SEM micrograph showing characteristics of the crystalline film.

Figure 4-11. b) EDXA spectrum showing elemental distribution of crystalline film on metal surface.

Figure 4-12. Batch bioreactor - formation of crystalline film on metal surface after exposure to bacteria. a) SEM micrograph showing a transition zone from biofilm to crystalline film.

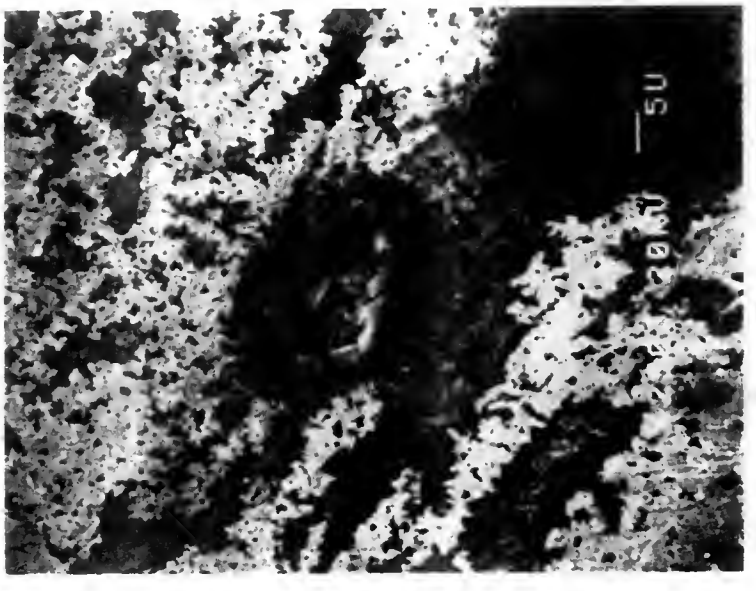


Figure 4-12. b) SEM micrograph showing a deteriorated polymeric biofilm structure and pitting after bacteria had died.

The Electrochemical Cell

The electrochemical cell was basically a modified batch bioreactor equipped for making electrochemical measurements integrated with a data acquisition system as described in Figure 3-3, page 93. Using this set-up, several experiments were performed excluding bacteria and their results were used to select the parameters for the runs with the final modified electrochemical cells. The theory for all electrochemical cells is described in Capabilities of the Triad Cell, page 198.

The experiments included qualitative and quantitative determinations of hydrogen production by some metals, free corrosion potentials at different metal-surface polishing grades, a galvanic series of some metals in the mineral electrolyte solution, bacterial growth characteristics, and hydrogen uptake by some hydrogen oxidizing bacteria.

Table 4-3 shows the results of hydrogen production by three different metals in the mineral electrolyte solution. Mg, the most active, produced hydrogen almost immediately and in 1 hour reached an average hydrogen concentration in the head space of 24.4%. For the other two metals, Zn and SA106 steel, hydrogen was not detected even six hours after the test was begun. After one day, hydrogen production was appraised by the head space pressure which was measured for several

Table 4-3. Hydrogen production by filings of Mg, Zn, and SA106 steel in mineral solution in the absence of bacteria after three days.

		Day 1		Day 3
Metal	wt, gr	Press	H_2 conc	Press
		mm Hg	%	mm Hg
Mg(1)	0.5808	230	29	1870
Mg(2)	0.5811	410	21	1545
Mg(3)	0.5807	12	23	1680
Zn(1)	1.6847	90	0	170
Zn(2)	1.6843	130	0	190
Zn(3)	1.6842	146	0	210
SA106(1)	1.2199	149	0	160
SA106(2)	1.2195	147	0	150
SA106(3)	1.2193	124	0	135
CONTROL(1)	0	178	0	175
CONTROL(2)	0	183	0	181
CONTROL(3)	0	178	0	175

days. A noticeable increase in the head space pressure, for the case of steel, started after three days.

Table 4-4 presents the free corrosion potentials of SA106 steel metal coupons polished at diffent grades in the mineral electrolyte solution. Results indicated that the free corrosion potential was not affected substantially in that range of surface polishing grades tested. For the purpose of this research, the 600 grid polish was selected. This grade

of polish provided a combination of a little roughness and a fine surface.

Table 4-4. Free corrosion potential of SA106 steel coupons at different surface polishing grades.

Polishing grade grid #	Free Corrosion Potential Volts (Ag/AgCl)
180	-0.714
320	-0.684
400	-0.701
600	-0.680
1200	-0.680

Results of the free corrosion potentials of selected metals and alloys in the absence of bacteria are presented in Table 4-5. The free corrosion potentials are arranged in a galvanic series in order to compare their reactivities in the mineral electrolyte solution. Potential-time curves obtained during free corrosion potential measurements are shown in Figure 4-13.

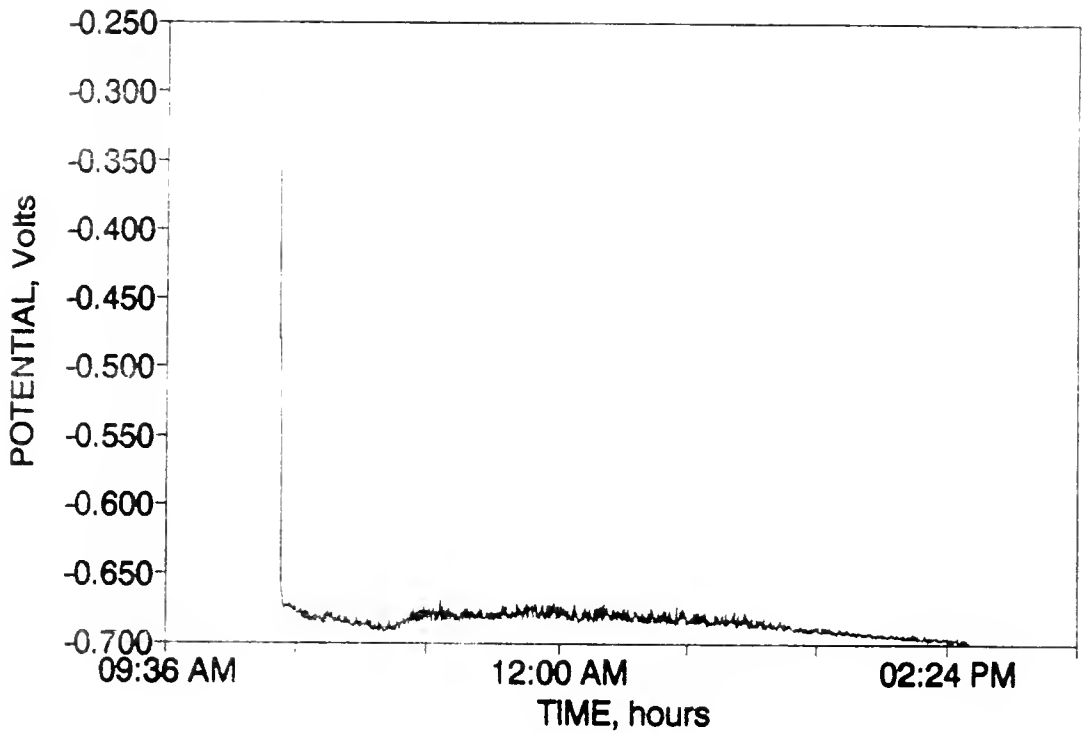


Figure 4-13. Potential-time curve in the absence of bacteria.

Measurement of the growth of pure cultures including absorbance, transmittance, density, and dry weight were performed to complement research in the development of the experimental system. Figure 4-14 shows the growth curve at 37°C for E. coli (JW111) in tryptic soy broth media by Difco. Results indicated that E. coli grew exponentially between 4 and 7 hours and reaches a constant growth after 8 hours. The usefulness of Figure 4-14 finds its place in experiments that require rapid interpretation such as results that require normalization using the weight of bacteria. This way,

Table 4-5. Galvanic series of selected metals and alloys in mineral solution at 30°C vs Ag/AgCl reference electrode in the absence of bacteria.

	Free corrosion potential
Metal coupon	Volts (Ag/AgCl)
Mg	-1.520
Zn	-0.960
Al	-0.750
Steel SX52(T)	-0.680
Steel SX70	-0.680
Steel SX60	-0.680
Steel SA106	-0.680
Steel SX42	-0.675
Steel SX42(B)	-0.670
Steel SX52(B)	-0.670
Steel SHP9430	-0.560
Steel SGRB	-0.550
Al 20	-0.540
Steel SS430	-0.170
Steel S430	-0.040
Cu-Ni 7030	-0.010
Cu	0.016
Ag-Zn	0.022
Cu-Ni 9010	0.025

bacteria dry weight is correlated with absorbance which is measured relatively rapidly. The drawback is that these growth curves are valid only for cases involving the same growth conditions.

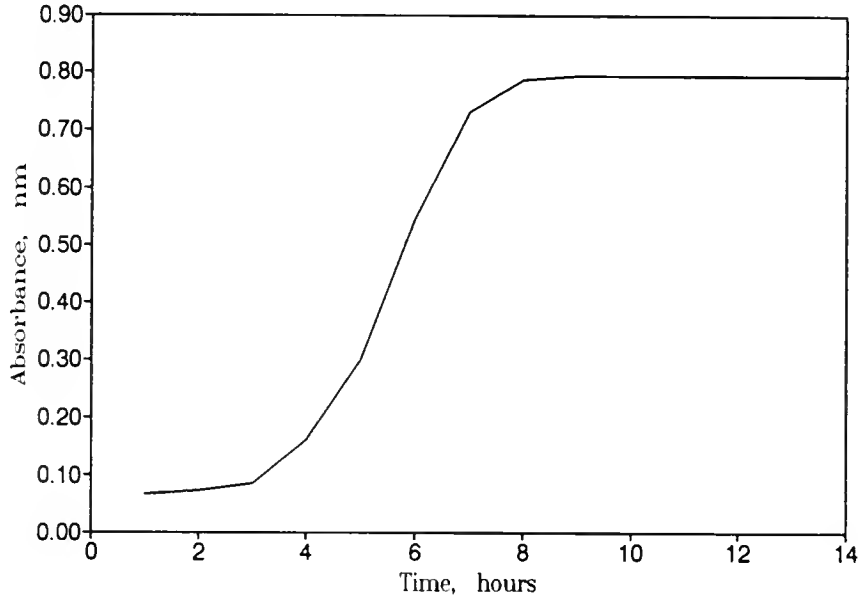


Figure 4-14. Growth curve for E. coli at 600 nm, 37°C, and trypticase soy broth medium.

The next two groups of experiments of this section consisted of: 1) determining the effect of media on E. coli during hydrogen uptake and 2) comparing the hydrogen uptake of different hydrogen oxidizing bacteria in the medium that E. coli had maximum hydrogen uptake. Table 4-7 shows that E. coli takes up over twice as much hydrogen in the mineral solution than in other media rich in organic nutrients. These results suggested that E. coli were forced to use more

hydrogen as the energy source in an anaerobic environment poor in nutrients.

Table 4-7. Effect of media composition on the hydrogen uptake by *E. coli* at 30°C during 6 hours.

Media	Hydrogen uptake, %	Bacteria dry wt, mg/L
Mineral Solution	24	350
Nutrient Broth	9	600
GLYE	11	500
Control	4	0

Table 4-8 indicates that despite the density of the different cultures their hydrogen uptake was very similar. These results agree with earlier experiments and has been suggested in the literature (Booth and Tiller, 1968).

Table 4-8. Bacteria hydrogen uptake capabilities in mineral solution at 30°C during 6 hours.

Bacteria	Hydrogen uptake, %	Dry weight, mg/L
D. desulfuricans	27	20
E. aerogenes	20	344
A. eutrophus	26	60
C. acetobutylicum	27	120
E. coli	24	350
Control	4	0

Single Flask Electrochemical Cell

The objective of this single cell was to measure the free corrosion potential of metals in the presence and absence of bacteria.

Using the setup indicated in Figures 3-6 and 3-7, a combination of experiments were performed to determine the effect of four different hydrogen oxidizing bacteria on the free corrosion potential of three different metals. Results of potential time curves showed that the presence of bacteria positively influenced the corrosion of the metals, except copper.

Figure 4-15 shows triplicates of potential-time curves of SA106 steel in the presence of A. eutrophus and the control, excluding bacteria. The potential differences during the tests were on the order of 150 mV. Figure 4-16 shows the free corrosion potential characterization of Mg in mineral solution in the presence of E.coli.

Metal coupons experienced a decrease in their free corrosion potentials when compared to the control experiments unexposed to bacteria. These results suggest the utilization of cathodic hydrogen by bacteria. Table 4-8 summarizes the results of this combination experiment using the single cell. An overall analysis of this experiment indicated that the free corrosion potential differences were independent of the type and amount of bacteria used. The free corrosion potentials

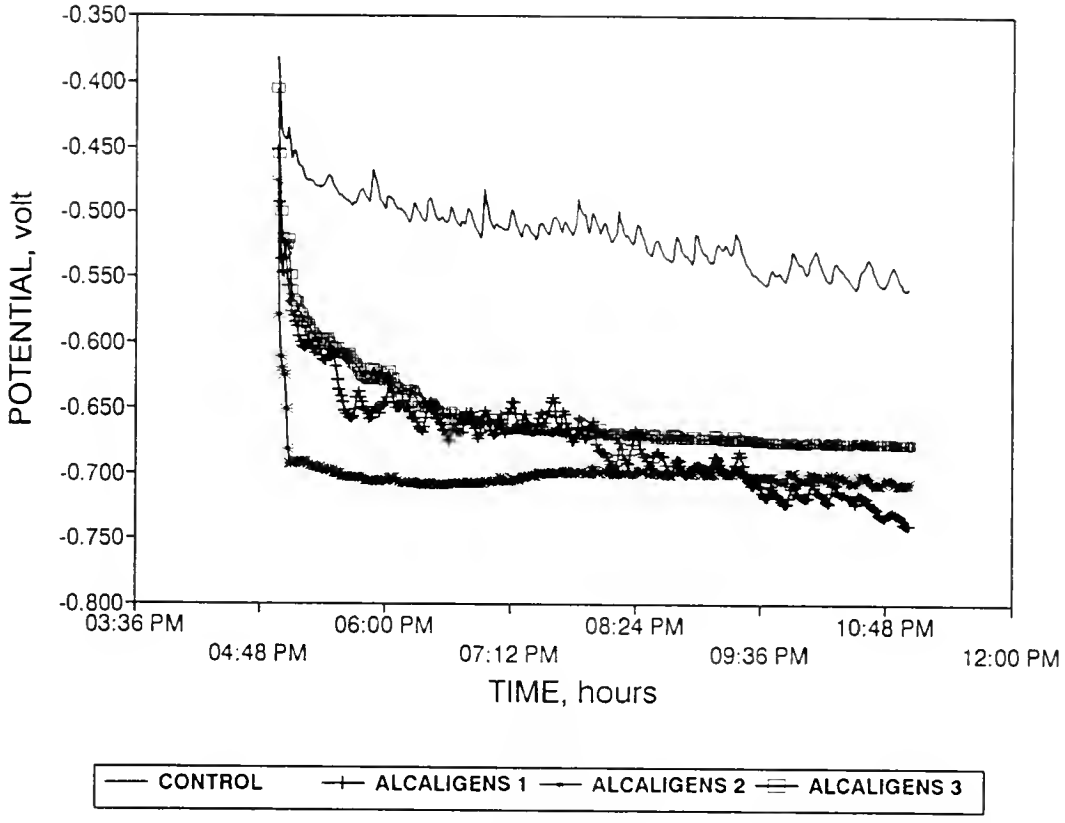


Figure 4-15. Potential-time curves for SA106 steel in the presence of A. eutrophus.

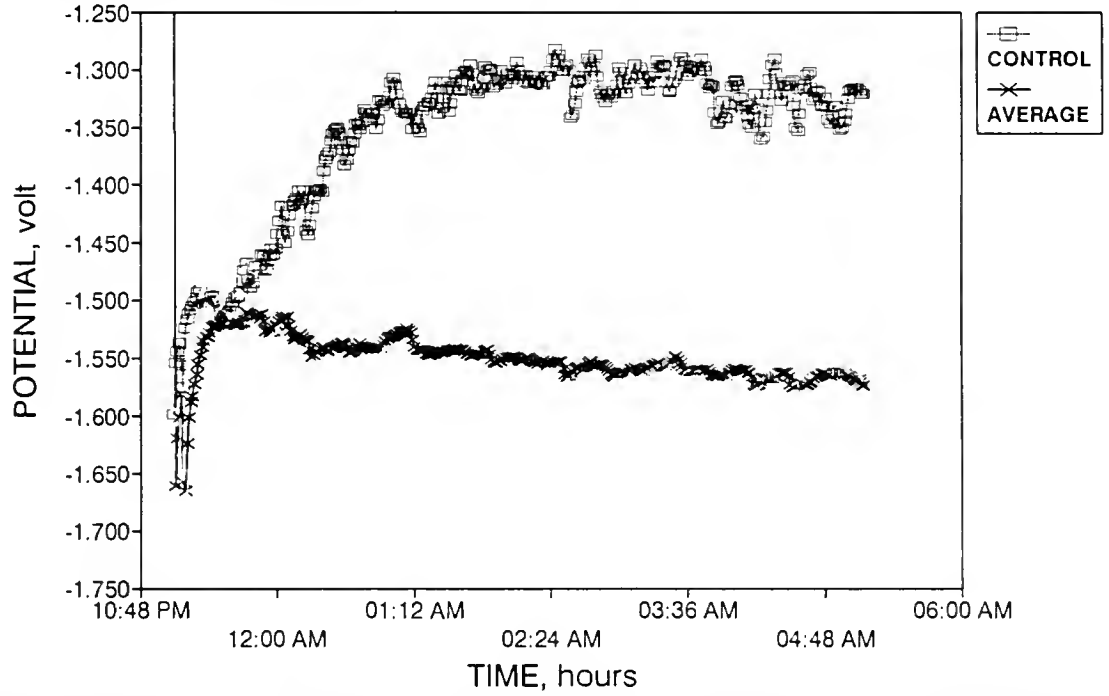


Figure 4-16. Potential-time curves for Mg in the presence of D. desulfuricans.

differences among the three metals tested were due to their reactivities which are related to their different capacities to produce cathodic hydrogen. The results with copper indicate that this metal might have been toxic to bacteria as suggested in the literature review section (Beveridge and Doyle, 1989).

Table 4-8. Effect of different bacteria on the free corrosion potential of Mg, Cu, and SA106 steel coupons in mineral solution and 30°C.

METAL	BACTERIA	FCP, Volts	delta FCP
Mg	A. eutrophus	-1.495	0.195
	E. coli	-1.475	0.175
	E. aerogenes	-1.545	0.245
	D. desulfuricans	-1.515	0.215
SA106	A. eutrophus	-0.655	0.095
	E. coli	-0.565	0.015
	E. aerogenes	-0.675	0.125
	D. desulfuricans	-0.645	0.085
Cu	A. eutrophus	-0.046	0.086
	E. coli	-0.005	0.035
	E. aerogenes	-0.047	0.087
	D. desulfuricans	-0.046	0.086

An important observation during the anaerobic experiments with the single flask electrochemical cell was the occurrence

of longitudinal cracks along the surface of the SA106 steel coupons which otherwise did not occur when metal coupons were in aerobic conditions. The cracks occurred after coupons were used several times and occurred in the direction the pipe was drawn. The cracks were first observed when unusual large bubbles appeared at the metal surface on the cracks. The cracks were easy to recognize when rust formed on them, after the test and out of the mineral solution, but were difficult to detect if the surface had been polished.

The discovery of this cracking phenomena and reports on some unaccountable cathodic hydrogen loss during electrochemical measurements in microbial corrosion experiments (Booth and Tiller, 1968), suggested that part of the cathodic hydrogen being produced was absorbed on the surface of the steel with concomitant production of hydrogen embrittlement. Figure 4-24, page 148, shows the effects of hydrogen embrittlement on SA106 steel.

Despite some encouraging results, the single cell was considered inappropriate to accomplish the objectives of the overall research for the following reasons: 1) the similarity of values of the free corrosion potentials for each metal, despite the amount and the bacterial species used, 2) the inability to handle metal toxicity as in the case of copper, 3) lack of confidence with the results of the potential-time curves, 4) the inability to measure corrosion current, the ideal parameter, 5) the possibility of suspended bacteria to

behave differently due to the imposed stress during centrifugation, and 6) instrumental failures that made difficult the maintenance of fixed experimental conditions.

In reference to the amount of bacteria inoculated, E. aergenes and E. coli outnumbered A. eutrophus by a factor of 12, and if compared to the amount of D. desulfuricans, the factor was even larger because of the very low dry weight that could be measured in a growing culture of the bacteria.

An overall review of the results and the components of the single flask electrochemical cell suggested that the source of the controversies might be due to the following factors: a) reference electrodes were calibrated individually instead of calibrated against each other b) The technique used to mount metal coupons in epoxy was not appropriate in some cases (some coupons experienced corrosion on the edges after the first run and others experienced galvanic coupling with the connecting copper wire), and c) the cracks that developed on the steel coupons could have influenced the results of the potential-time curves.

Dual Flask Electrochemical Cell

The dual flask is an improvement of the single cell. Its arrangement is described in Figure 3-5. This electrochemical cell included an additional flask (II) interconnected to the initial single cell, flask (I), through the head space in

order to allow bacteria to grow in their best growth media. This flask allowed bacteria to avoid direct contact with the metal and, consequently any metal effects such as toxicity. The new set-up permitted the utilization of the other channels of the data acquisition system for the measurement of other parameters such as pH, oxidation-reduction potential, and head space pressure.

Table 4-9 summarizes the results of five experiments that evaluated effects of bacteria and the addition of a terminal electron acceptor (fumarate) on the corrosion of SA106 steel. These results include calculation of average corrosion rates based on dissolved iron analysis. In addition, potential-

Table 4-9. Dual cell schedule of experiments and corresponding corrosion rates, using E.coli (JW111) and SA106 steel coupons.

Run		Bacteria			
#		Age	Dry wt	Fe conc	Corrosion
		hr	mg/L	(mg/L)x100	mg/dm^2D
1	control	-	-	7.4	47.0
2		6	46.7	6.3	40.0
3		15	63.3	7.5	48.0
4		23	59.4	7.4	47.0
5	fumarate	27	69.8	15.0	95.0

Note: Control run contained no bacteria.

time curves for these experiments are presented to show the effect of bacteria on free corrosion potential.

Figure 4-17 shows the effects of E. coli at different ages on the free corrosion potential of SA106 steel coupons. The potential-time control curve is included in every figure for comparison. Figure 4-18 indicates the corresponding dissolved iron profiles. Both curves show that the presence of E. coli, one of the most prolific bacteria in most aqueous environments, increased the corrosion of the steel. It is interesting to notice that this is the first time E. coli has been shown to influence the corrosion of metals; this hydrogen uptaking strain was able to cause an effect on the free corrosion potential at the onset of the corrosion process.

Figures 4-17a, b, c, and d and Figures 4-18a, b, c, and d represent the effect of E. coli, harvested by centrifugation and corresponding to 6, 15, 23 and 27 hours old and 46.7, 63.3, 59.4 and 69.8 mg/100 mL dry weight respectively. The bacterial effect on the potential-time curves and dissolved iron profiles was consistent with the rest of the research and influenced the corrosion of SA106 steel. In all experiments the potential-time curve was affected by the bacteria in the horizontal portion by 10 mV in the direction of the more active potential if compared to the control. The dissolved iron profile appeared to be unaffected for the first four experiments except for the case when fumarate was added. Results of the dual cell were still

inadequate to determine the influence of bacteria in the corrosion of steel because of the insignificant potential differences and the inaccuracy of the iron analysis from stagnant fluids.

Figures 4-17d and 4-18d represent probably the most important finding from an experiment done with the dual cell. They show the effect of fumarate, a terminal electron acceptor. Results of the potential-time curve show a dual effect on both segments of the curve if compared to the control. The effects are comparable to the observed in the cases of an induced mass transfer and a bacterial effect combined. Results of the dissolved iron profile showed a two fold increase after the addition of 40 mM of fumarate to the single cell. These results suggested that the reducible substrate activated the bacteria hydrogenase system during their uptake of cathodic hydrogen. During the dual cell runs other parameters including pH, oxidation-reduction potential, and head space pressure were measured in order to determine their trend. All experiments had a similar trend: pH increased during the first 0.5 hour 0.3 pH units and then stabilized at a rate of 0.05 pH units every 2 hours. Oxidation-reduction potential of flask I decreased linearly 140 mV during the first 3 hours and oxidation-reduction potential of flask II increased exponentially 140 mV during the same time period. Head space pressure decreased very

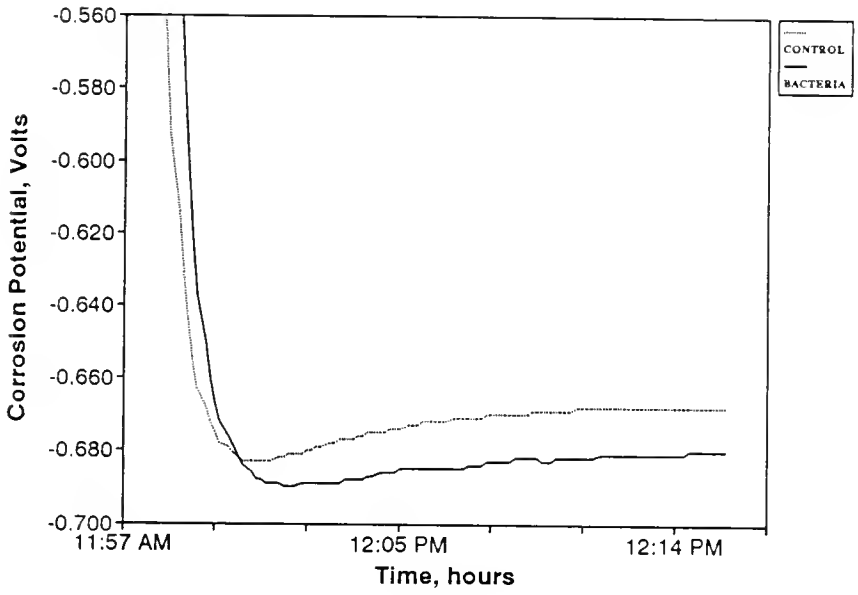


Figure 4-17. Dual cell run potential-time curves. a) Effect of E. coli, 6 hr old .

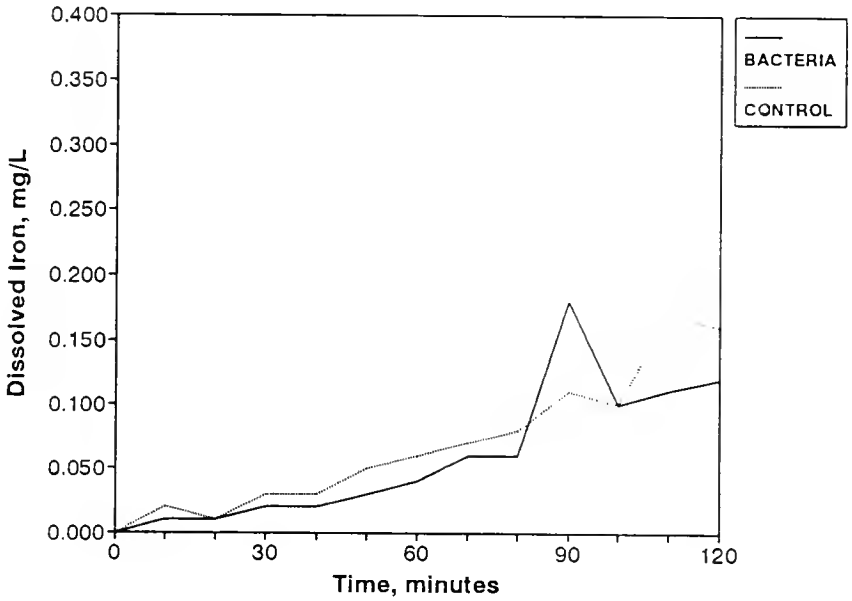


Figure 4-18. Dual cell run dissolved iron profiles. a) Effect of E. coli, 6 hr old.

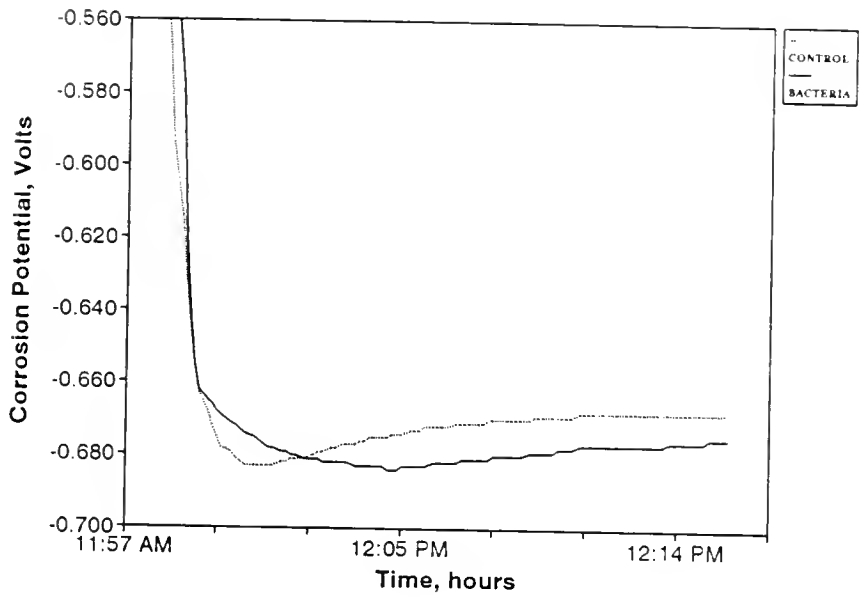


Figure 4-17. b) Effect of E. coli, 15 hr old .

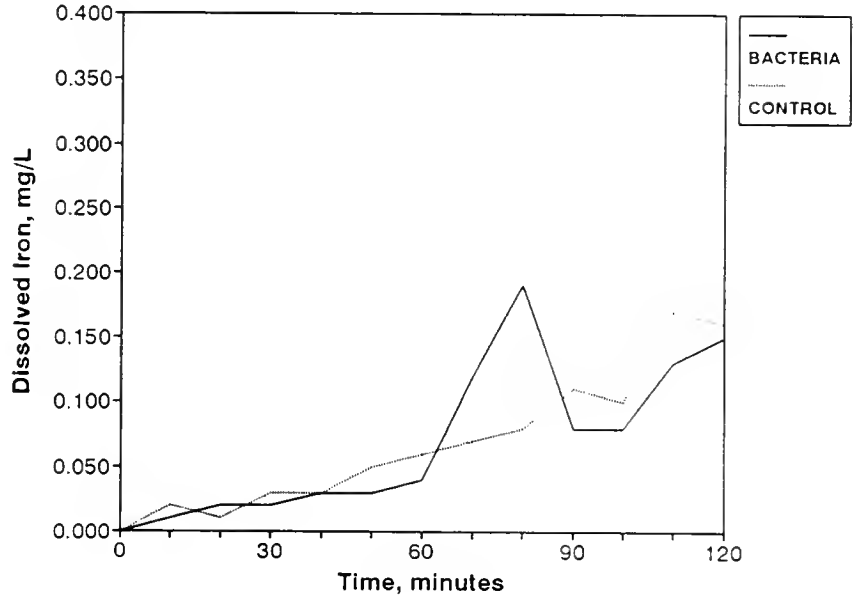


Figure 4-18. b) Effect of E. coli, 6 hr old.

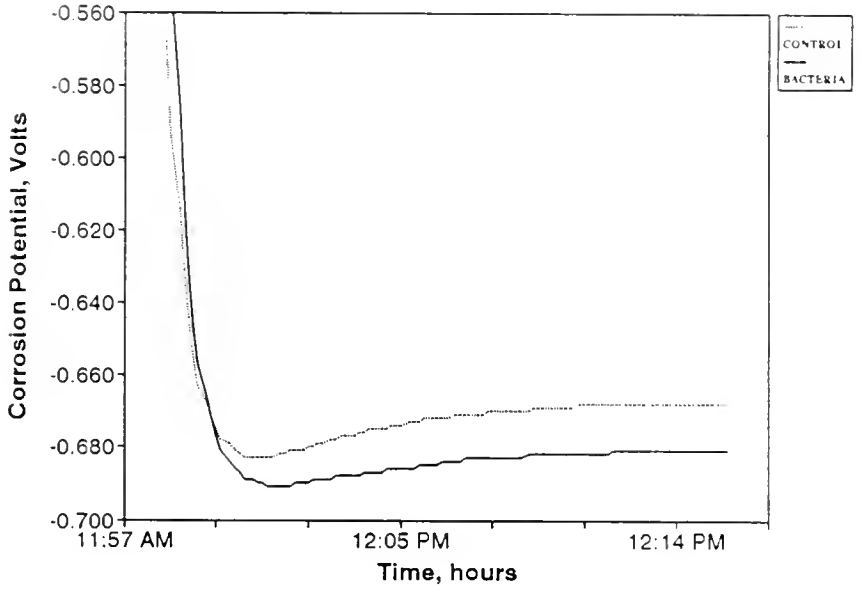


Figure 4-17. c) Effect of E. coli, 23 hr old .

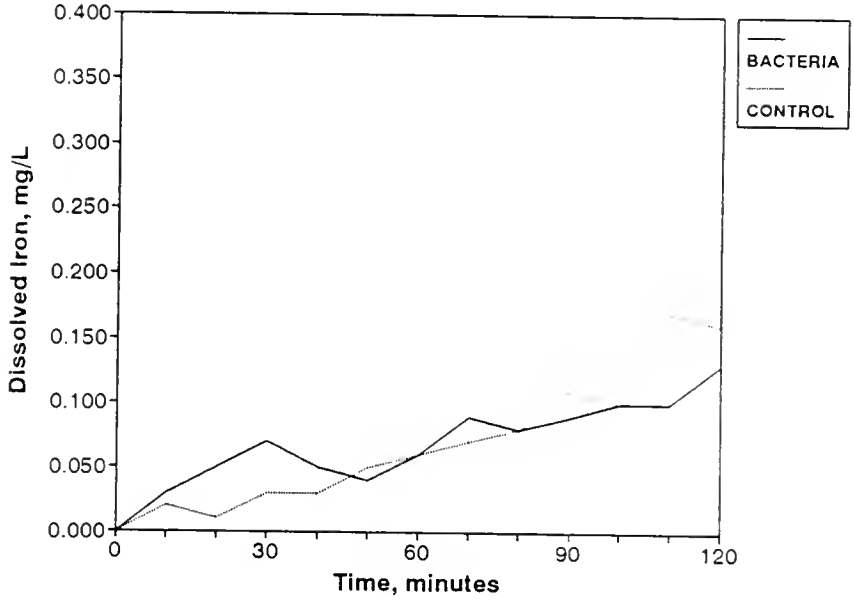


Figure 4-18. c) Effect of E. coli, 23 hr old.

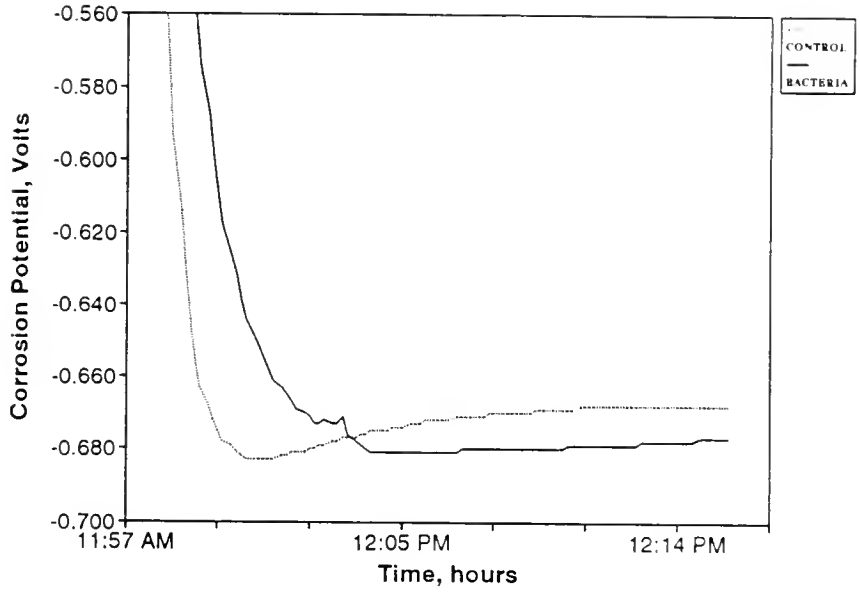


Figure 4-17. d) Effect of E. coli, 27 hr old, and fumarate .

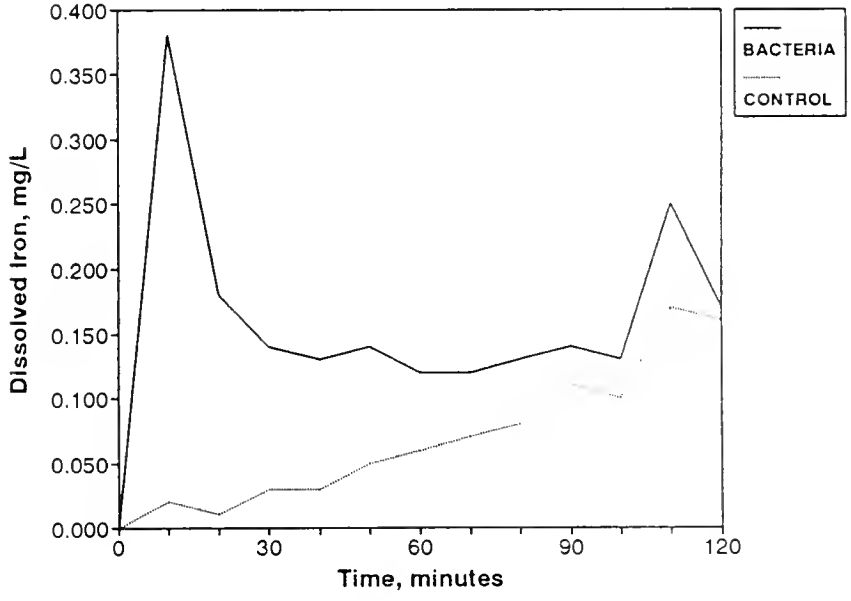


Figure 4-18. d) Effect of E. coli, 27 hr old, and fumarate.

slowly until samples were taken, then it decreased proportionally to the sample volume drawn.

During the dual cell runs it was observed, as in the single cell runs, that SA106 steel coupons developed longitudinal cracks along their surfaces after coupons were used more than four times. These observations confirmed the development of cracks and clearly suggest the occurrence of hydrogen embrittlement. Results are shown in Figure 4-24, page 188.

An overall review of the dual cell experimental results indicated that the dual cell was also inadequate to quantify the effect of bacteria during the corrosion of steel. The reasons considered here were the same ones presented for the single cell. The insignificant potential difference resulting from the activity of the bacteria led to abandoning the idea of attempting to correlate the potential differences with their biological corrosion effect. Fortunately, the effect of fumarate on bacterial activity encouraged the search for a new idea to incorporate the measurement of a corrosion related current parameter into the dual cell which resulted in the triad flask electrochemical cell.

Triad Flask Electrochemical Cell

The purpose of the triad flask electrochemical cell was to incorporate the measurement of a corrosion related current,

into the dual cell to determine the influence of bacteria during the corrosion process. A detailed schematic of the triad cell is presented in Figure 3-6. The triad cell included a third flask (III) similar to flask I, except bacteria was not added to it. The set-up allowed the measurement of a differential corrosion current (DCC) that could be correlated to the corrosion influenced by bacteria.

The triad cell is comparable with a microbial corrosion syatem and should be able to measure the utilization of cathodic hydrogen by bacteria. The head space connection between flasks I and II could be associated with the hydrogen diffusion restriction between the bacteria and the metal through a membrane or biofilm. Flask II could be associated with a growing environment such as the one found within a biofilm where bacteria could perform at optimum capacity. Here bacteria could be protected from toxic effects and grow under their optimum conditions, and could control the corrosion reaction mechanisms (Little and Wagner, 1993; de Beer et al., 1992). The triad cell also allowed the use of a resting cell either in flasks I or III and the measurement of a differential corrosion current (DCC) between flasks I and III that may be correlated with the bacterial utilization of cathodic hydrogen. Furthermore, the triad cell could allow the measurement of cathodic hydrogen utilization by bacteria indirectly via metabolism of a terminal electron acceptor such as fumarate. Additionally, the effect of bacteria on the

corrosion of metals might also be calculated from dissolved iron differences between flasks I and III. In general the triad cell could offer several alternatives to determine the biological component of corrosion.

A total of six experiments were performed using the triad cell. The first four shown in Table 4-10 include DCC measurements with an electrometer. On the last two experiments a zero resistance ammeter was used in order to improve inconsistent current measurements obtained with the electrometer.

Table 4-10. Triad cell schedule of runs and related corrosion using E. coli (JW111) and SA106 steel coupons.

	Run	Bacteria			
#		Age	Dry wt	Fe conc	Corrosion
		hr	mg/L	(mg/L)x100	mg/dm^2Day
1	control	-	-	0.75	4.7
2		8	67.5	0.42	2.7
3	fumarate	20	64.3	2.58	16.3
4		>6D	71.9	0.08	0.5

The initial four experiments evaluated the effects of E. coli on the corrosion of SA106 steel through changes of the shape of the potential-time curve, the dissolved iron profile, and the DCC-time curve. The last two experiments portray an

exaggerated case of hydrogen uptake and hydrogen production using SA106 steel, Cu, and Mg coupons.

Results of Figure 4-19 and Figure 4-20 show the effect of E. coli on the anaerobic corrosion of SA106 steel in mineral solution. Potential-time curves and dissolved iron profiles indicate that the presence of bacteria influenced the corrosion of the steel.

Figures 4-19a and 4-20a indicate the effect of bacteria, harvested after 8 hr, on the corrosion of SA106 steel coupons. Results showed a shift of 5 mV if compared to the control, in the direction of the more active potential, on the horizontal portion of the potential-time curve. Dissolved iron analyses showed an average corrosion rate of 2.7 mg/dm^2D and the DCC indicated an average current of 215 nA. Potential-time curve and dissolved iron profile of the control run showed characteristics similar to their equivalent dual cell curves. The magnitude of the differential corrosion current (DCC) results was very small for the control run, on the order of 1 nA as expected. Figures 4-19b and 4-20b show the effect of bacteria harvested after 20 hours including the addition of 40 mM of fumarate. Results showed a dual effect on the shape of the potential-time curve similar to the corresponding run of the dual cell (Figures 4-17d and 4-18d). The presence of the terminal electron acceptor increased corrosion rate over three times the value of the control run, to an average corrosion rate of 16.3 mg/dm^2D calculated from the dissolved iron

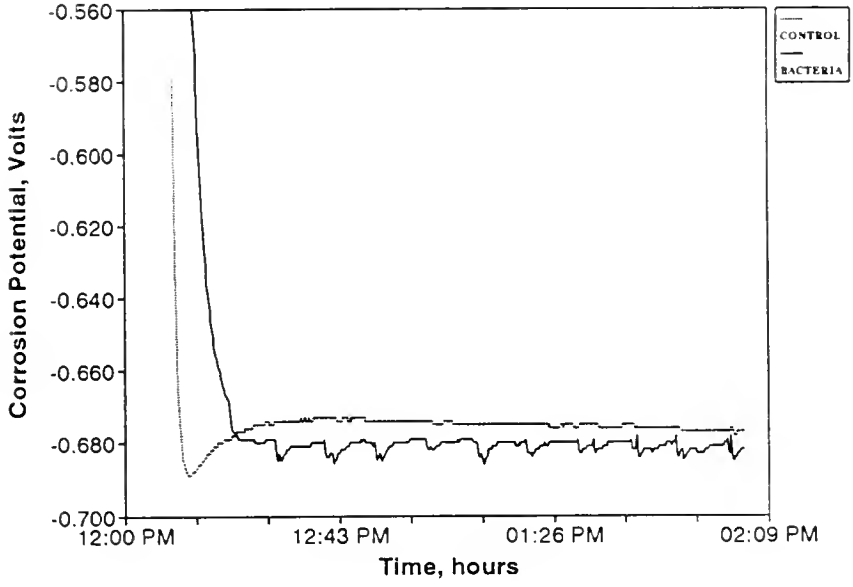


Figure 4-19. Triad cell potential-time curves. a) Effect of E. coli, 8 hr old.

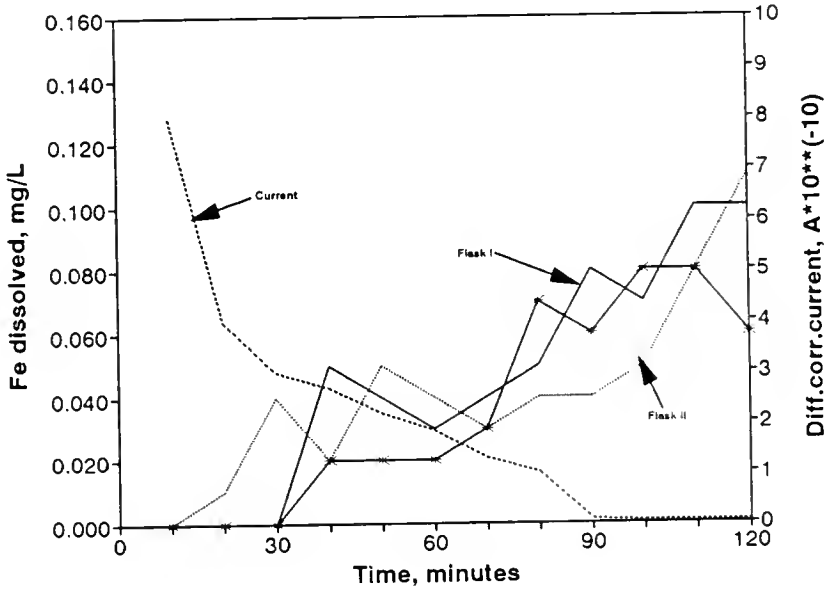


Figure 4-20. Triad cell dissolved iron profiles. a) Effect of E. coli, 8 hr old.

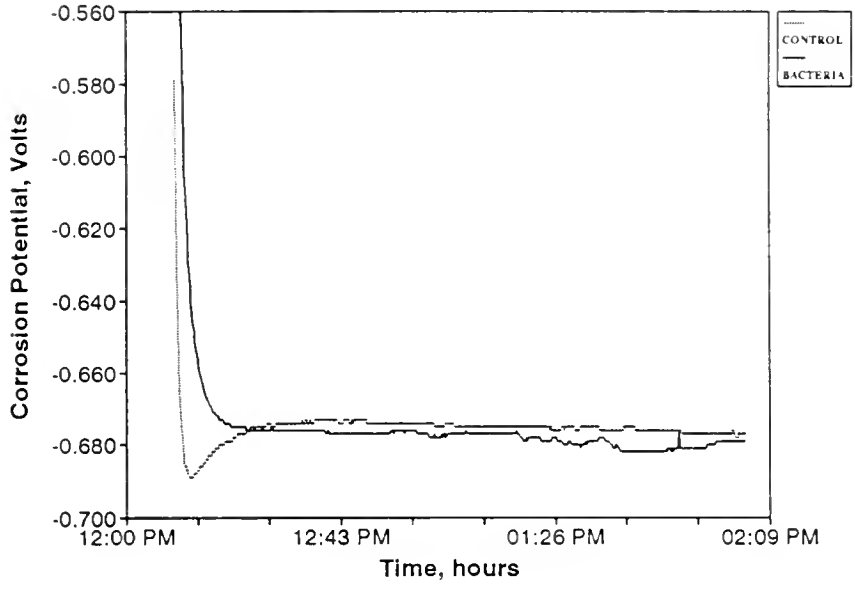


Figure 4-19. b) Effect of E. coli, 20 hr old and fumarate.

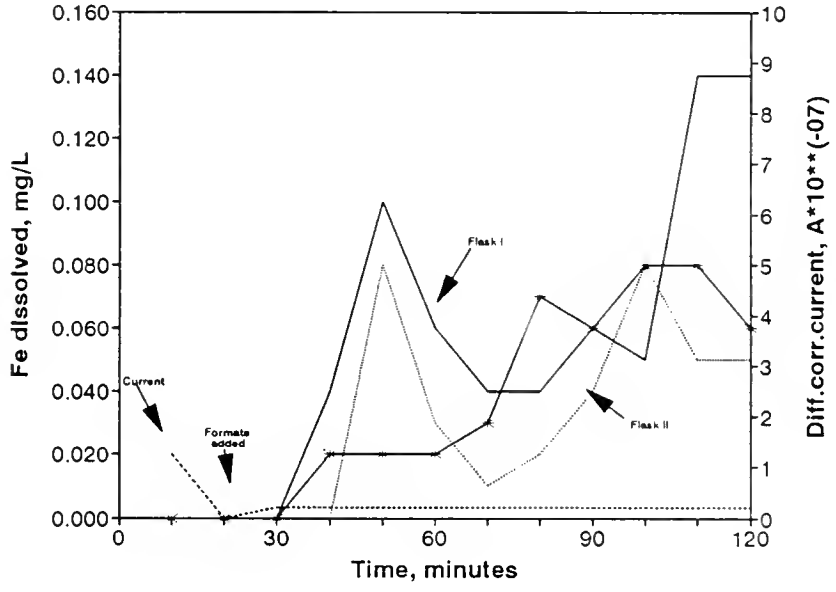


Figure 4-20. b) Effect of E. coli, 20 hr old and fumarate.

profile. A similar result was observed with the DCC that jumped 20 nA as soon as the fumarate was added. This important observation suggested once again that the addition of fumarate

increased the activity of the bacteria and hence the microbial corrosion process. Figures 4-19c and 4-20c show the effect of bacteria harvested after 6 days. Results indicated that potential-time curve had a similar effect as with bacteria harvested within 20 hours. However, the corrosion rate calculated from the dissolved iron profile was very low, 0.5 mg/dm^2D.

The DCC was not accurately measured due to the continuous inconvenients with the eletrometer calibration and chart recorder signal noise. In order to improve DCC measurements, a home made zero resistance ammeter was implemented and instrumented to the data acquisition system. The last two experiments were performed using the new device.

Overall results of the first four experiments indicated that potential-time curves, dissolved iron profiles and DCCs were not significant; due to the low potential differences produced by the presence of bacteria, the unreliable dissolved iron analysis as a result of the stagnant conditions of the triad cell, and the inaccuracy of measurements provided by the electrometer. The first of the last two experiments of the triad cell depict the exaggerated conditions of hydrogen

uptake using SA106 steel coupons as in previous experiments. The purpose of this test was to determine the sensitivity of

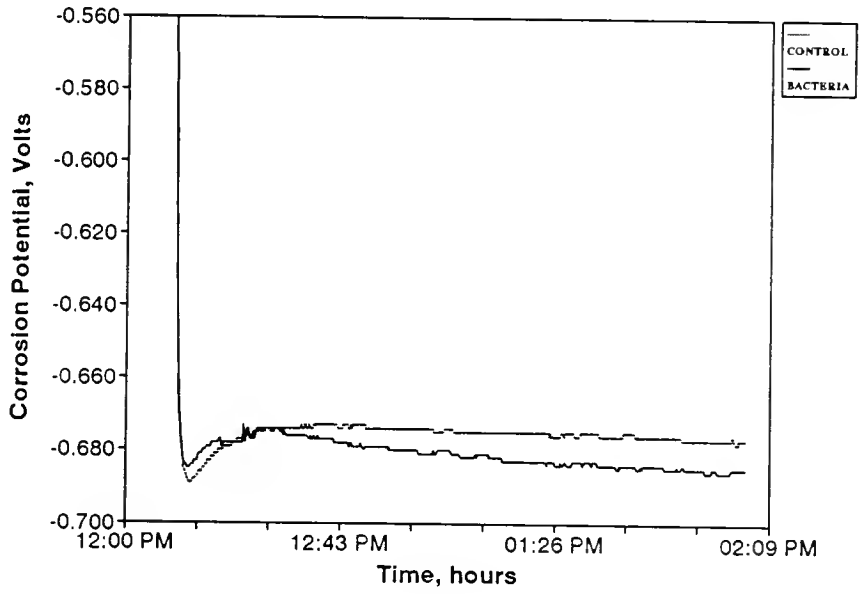


Figure 4-19. c) Effect of E. coli, > 6 days old.

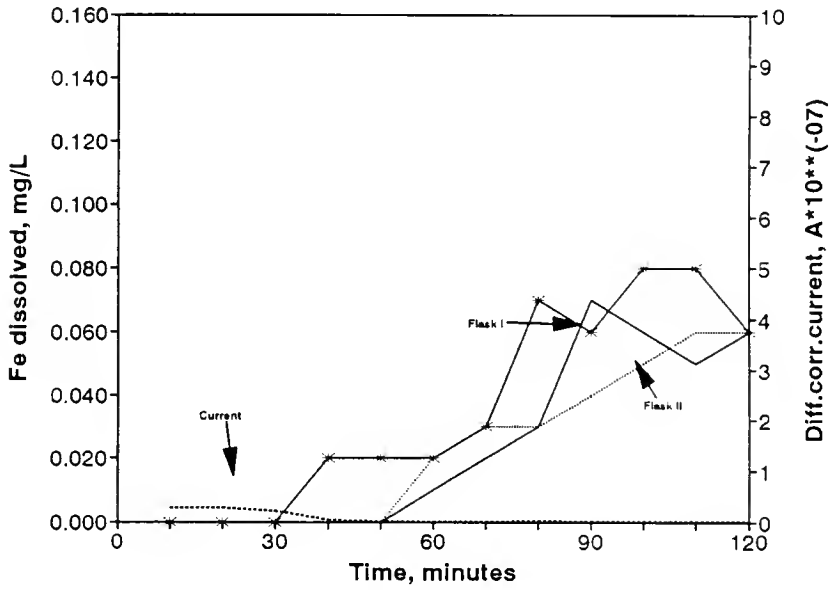


Figure 4-20. c) Effect of E. coli, > 6 days old.

the triad cell by applying artificial vacuums to flask I and injecting volumes of hydrogen gas to flask III. The artificial vacuums represented larger than normal hydrogen uptakes by bacteria.

Figure 4-21 shows the behavior of the potential-time and differential current-time curves under forced conditions of hydrogen uptake and hydrogen injection. Potential I represents the potential of the working electrode in flask I, potential III represents the potential of working electrode in flask III, and current represents the differential corrosion current (DCC).

As indicated in the figure, two initial vacuums, of 3mm Hg each, were applied to flask I. These forced vacuums were equivalent to exaggerated hydrogen uptakes by bacteria. Results indicate that none of those vacuums produced any effect either in the DCC curve or in any of the two potential-time curves for flasks I and III. However, when two 50 mL of hydrogen gas were injected in flask III, some changes were observed on the curves. The initial volume injected caused an instantaneous decrease in the DCC in the order of 1 μA and the second injection increased potential III by 12 mV and the DCC stabilized. Then, after 20 minutes, potential III returned to its original value and the DCC level off until the end of the test.

The second of the last two experiments of the triad cell represents a modified Daniel cell, a very early form of

battery which consisted of Cu and Zn electrodes in a solution of their salts except in this experiment the Zn electrode was replaced by a Mg electrode in order to maximize the cell voltage and consequently the differential corrosion current

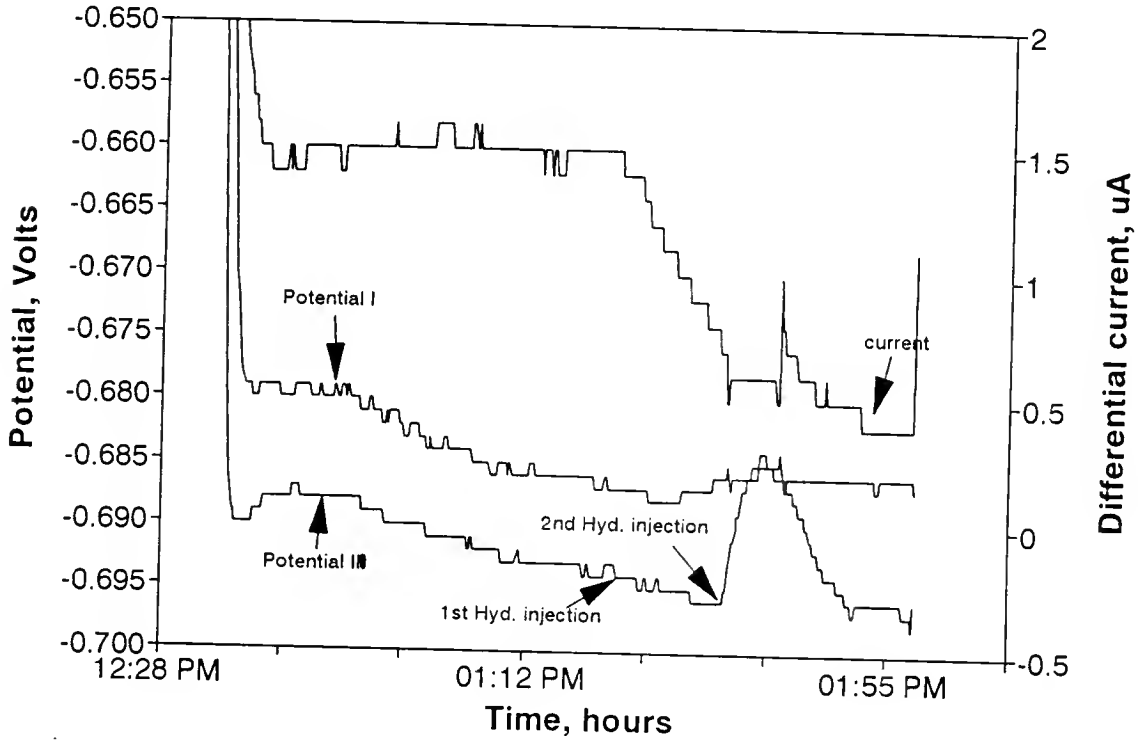


Figure 4-21. Triad cell artificial hydrogen uptake test.

(DCC). The electrolyte solution used in this last experiment was the mineral solution utilized throughout this investigation. The purpose of this experiment was to examine the measurement capabilities of the triad cell by comparing it to a known electrochemical model cell as the Daniel battery that produces a significant amount of current. Also, attempt to explain the controversial measuring difficulties of

previous triad cell runs in anaerobic conditions and further demonstrate the capabilities of the triad cell.

Figure 4-22 shows the results of forced vacuums of 3 mm Hg applied to flasks I and III and their effects on the DCC and electrode potentials during the corrosion process of Mg. The forced vacuums represented artificial hydrogen uptakes. In this cell the SA106 steel electrodes were replaced by the Cu and Mg electrodes. The first vacuum, applied to the flask containing the Cu electrode, produced an increase in the Mg potential on the order of 200 mV and a decrease of 30 μA in the DCC. The Cu potential remained almost unchanged for the rest of the experiment. A second vacuum applied 20 minutes later produced an additional increase of 300 mV in the Mg potential and a decrease of 5 μA in the differential corrosion current. A third vacuum was later applied to the flask containing the Mg electrode produced an increase in the order of 2 μA in the differential corrosion current. After 10 minutes a fourth vacuum was applied to the flask containing the Mg coupon and significant changes were observed: the Mg potential decreased to its original potential, by 500 mV, and remained constant until the end of the test; and, the DCC increased by 35 μA within 5 minutes, then decreased steadily by 15 μA to a constant value of 35 μA until the end of the experiment.

Despite the evidence of experimental results that demonstrate microbial utilization of cathodic hydrogen during

the corrosion of SA106 steel, the inaccuracy and inconsistency of some of the results carried over from previous experiments and the sensitivity of the electrometer and the zero resistance ammeter did not provide accurate enough information to quantitatively determine the biological component of corrosion. However, results suggest that the triad flask electrochemical cell may be used to study the anaerobic utilization of cathodic hydrogen, provided some improvements be made in the following areas: measurement of the DCC, data acquisition system, rigidity of the cell, mechanical handling of metal coupons, gas and liquid sampling, anaerobic conditions, and water bath.

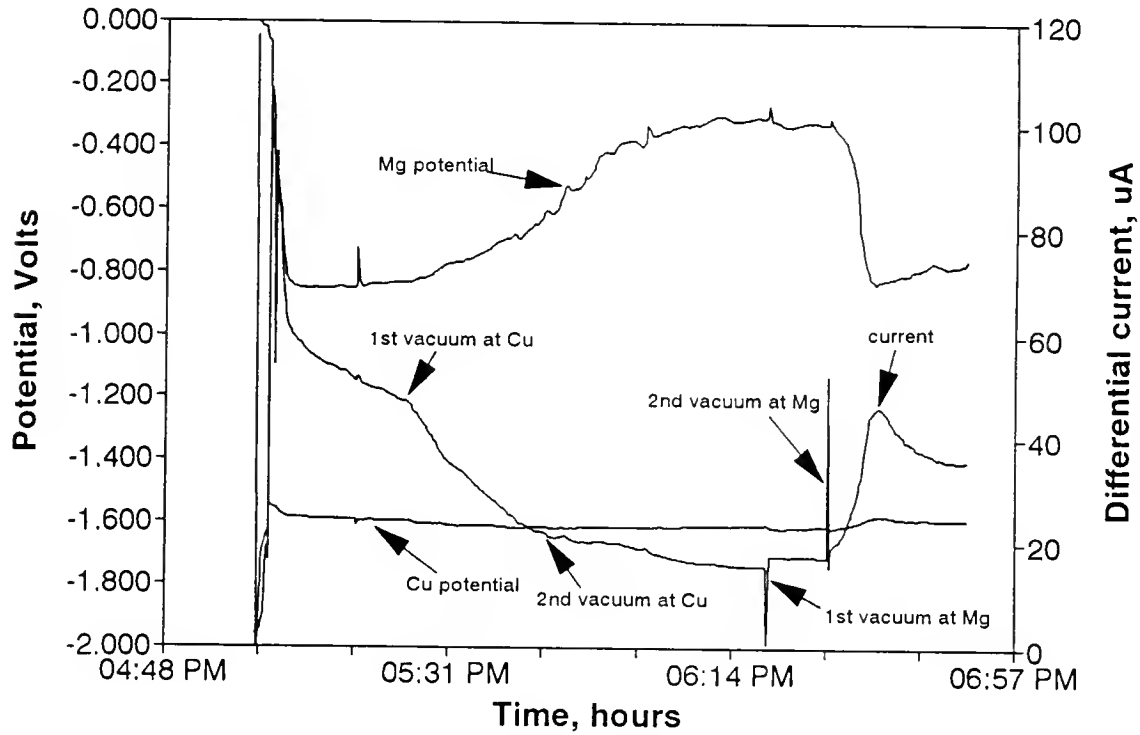


Figure 4-22. Artificial hydrogen uptake in a triad cell using Cu and Mg electrodes.

Final Discussion

In general this investigation presents a new approach to studying anaerobic microbial corrosion. The research shows evidence of metal deterioration by bacteria using two new flowthrough and batch bioreactors. It shows proof of the occurrence of hydrogen embrittlement and the effect of a terminal electron acceptor in the activity of bacteria during corrosion. A new microbial corrosion mechanism is proposed to provide a better insight to the overall understanding of this complex phenomena.

Figures 4-1 through Figure 4-8 show evidence of anaerobic corrosion of carbon steel by a known bacteria triculture, using the flowthrough bioreactor. Figures 4-9 through Figure 4-12 show evidence of microbial corrosion by single and combinations of the bacteria triculture, using a new batch bioreactor system. The batch bioreactor was able to reproduce microbial corrosion within one day and resulted practical to study the on-set of the corrosion process. This bioreactor allowed visual inspection of coupons and was less prone to bacterial contamination. In addition, further results of the batch bioreactor indicate that head space gas composition affected the bacteria/metal/fluid system. The presence of a black precipitate at the metal surface and in the bulk of the fluid was determined to be caused by both the activity of the

bacteria and the composition of the media. Other results suggest bacteria metabolism of nitrogen, hydrogen and methane.

The batch bioreactor was further implemented to resemble inside conditions of gas transmission pipelines, incorporate electrochemical measurements, and improve control of fixed parameters. The new experimental system became the electrochemical cells. An effort was made to set the stage for the study of microbial corrosion at its on-set. This included the use of suspended bacteria cells harvested by centrifugation instead of inoculum from their standard growth media and the use of the mineral electrolyte solution that provide the environmental conditions for hydrogen evolution from metals. The bacteria triculture used in early experiments was effective in the reproduction of microbial corrosion but it was not appropriate to for this study because of the concomitant effect of many variables resulting from their use. Effects such as the production of extracellular polymeric materials, bacteria age, ecological succession of bacteria involved, environmental growth conditions for different bacteria. The mineral solution developed did not allow the formation of a biofilm within two days because of the absence of organic nutrients that enhance the production of polysaccharides (Wachenheim and Patterson, 1992) and provided the chemical conditions for hydrogen evolution. Suspended cells were used to avoid retention of metabolites and other substances usually present in the growth media

contained in inoculum from standard medium. Iverson (1968) demonstrated that resting cells of Desulfovibrio vulgaris and Desulfotomaculum orientis grown in standard medium act as depolarizing agents of mild steel. However, his experimental procedure was criticized because it was found that these microorganisms often retain FeS in their cell walls (Tiller, 1982).

Microorganisms in general grow under many different environmental surroundings. However they can also adapt, and bacteria would make differential changes for a different physiological state. Those changes could be negative such as diminishing their biosynthetic activities, stopping their reproduction capabilities, and increasing their catabolic processes with production of secondary metabolites; or could be positive such as improvement of their growth and regaining optimal physiological conditions as under a biofilm.

Researchers have found different pH profiles inside biofilms than in the bulk of the liquid (Wagner and Little, 1993). pH changes have been reported on bacteria to resist heavy metal toxicity because of their effects on the hydrolyzed forms of the heavy metals and their toxic effects (Collins and Stozky, 1992). It has been reported also that different bacteria species have different metal-oxidizing capacities (Corstjens et al., 1992).

The utilization of cathodic hydrogen by bacteria is directly related to their optimal growth conditions which are

not always the most favorable for hydrogen formation (Cord-Ruwish and Widdel, 1986), low pH values stimulate the proton hydrogen equilibrium in the direction of hydrogen formation.

Due to the electrochemical nature of this research and the need for simplification, one bacterium was selected to continue this research. The selection of the proper bacterium used in the electrochemical experiments was based on availability, ease of growth, amount of bacteria harvested at the log phase, and hydrogen uptake capabilities. A literature search on the hydrogenase enzyme, also suggested the bacteria contain the proper hydrogenase. After contacting some researchers in the area, it was found that Dr. K.T. Shanmungam from the University of Florida had a mutated strain of E. coli (JW111), containing only hydrogenase for hydrogen uptake. Since this strain met all the above requirements, it was the best selection. It is a facultative anaerobic microbe that reaches maximum growth after 8 hours, as compared to D. desulfuricans that requires over 70 hours. When harvested by centrifugation at 10,000 g it yields a dry weight cell concentration in the range of 70 mg/ 100 mls of culture, compared to d. desulfuricans and C. acetobutylicum that yield a dry weight bacterium concentration around 5 mg/100 mls. In addition this particular bacteria has been tested in hydrogen-fumarate medium and results indicate that the bacteria uses 100% of fumarate during hydrogen uptake.

During all experiments standardization procedures were carefully followed for all electrochemical and analytical measurements in order to attain reliable measurements. A series of experiments were performed using the experimental setup described in Materials and Methods to assure the satisfactory performance of the measuring instruments. The experiments consisted of calibrations of the instruments and experimental runs following recommended standard procedures.

The corrosion of a metal in an aqueous environment is well recognized as a surface electrochemical phenomenon where part of the metal, represented as the anode, is oxidized and dissolved into the solution. The balancing cathodic reaction is composed of simultaneous reductions of some components of the environment (mineral solution) affecting the metal. The dissolved metal ions may either precipitate as insoluble compounds that may be loose or bulky or may attach firmly to the surface.

The role of the bacteria, on the other hand, is not as well defined as the role of the metal and the aqueous solution, since it may participate directly or indirectly in one or both of the electrochemical reactions on the metal, thereby influencing the reaction.

Once the SA106 steel coupon is immersed in the acidic electrolyte solution, the dominant reaction is iron dissolution which leaves behind an excess of electrons:

$$Fe \text{ -----> } Fe^{++} + 2e^{-} \qquad (1)$$

the excess electrons are consumed by the balancing reaction at cathodic sites. In the anaerobic set-up of the electrochemical cells the dominant balancing reaction is the reduction of hydrogen:

$$H_3O^+ + e^- \text{-----> } 1/2\ H_2 + H_2O \quad (2)$$

At pH < 7, which is the case of the mineral solution, hydrogen evolution usually predominates in the absence or presence of oxygen.

The theory of cathodic depolarization predicts that a protective hydrogen envelop forms on the metal surface and polarizes it. However, this was not the condition for the metals tested. In the case of experiments involving SA106 steel, Zn, and Mg, results shown in Table 4-3 indicate that the amount of cathodic hydrogen production is dependent on the type of metal. Furthermore, it depends also on the surface area of the metal, electrolyte composition, pH, and surface orientation. In all cases a protective hydrogen film never formed. In the Mg coupons the hydrogen bubbles evolved from the surface for several days and in the Zn and steel coupons fewer bubbles formed but left the surface also. In the case of metal filings of the same metals results show that for Mg, hydrogen bubbles emerge from the metal continuously for a period of time undetermined, then, after a while the flow of bubbles apparently stops for a moment and then larger bubbles arise bringing the metal filings with them. A similar event happened with the Zn filings but over longer time periods.

The steel filings produced bubbles after 3 days and required some shaking to observe hydrogen evolution. It was observed that the lower the pH used, as a result of carbon dioxide diffusion into the mineral solution, the more hydrogen evolved from the metal coupons. Initial experiments had the metal coupons oriented horizontally right below the tip of the reference electrodes. This practice was a source of faulty potential measurements because it allowed hydrogen bubbles to fill up the tip of the electrodes. Further experiments used coupons reoriented vertically as shown in Figure 3-4.

After the dual flask experiments by Belay and Daniels (1990), Rajagopal and LeGall (1989), and the implication of a phosphate compound in the corrosion process (Weimer et al., 1988); it has been suggested that there might be some component in the media that interact with the metal to induce the production of hydrogen. Bryant and Laishley (1993) were puzzled with the results of the dual flask experiment of Belay and Daniels, as how the interaction of the metal and the media could generate enough hydrogen to actually promote corrosion in view of the cathodic depolarization theory. The theory states that after metal is polarized, it requires the bacteria to depolarize it. They suggested that the interaction of phosphate with the steel promotes the release of cathodic hydrogen by an unknown mechanism. They based their declaration on experiments that demonstrated that an increased phosphate concentration in the media influenced the corrosion

of carbon steel and the production of black iron phosphates such as vivianite.

The evolution of hydrogen may be explained from a chemical point of view. Equation (2) above shows the overall cathodic reaction via the formation of atomic hydrogen which may be absorbed in the metal surface. Normally, molecular hydrogen is produced by a combination reaction which is slow and often becomes the rate limiting step in the corrosion process (Miller, 1981).

$$2\ H \text{ -----> } H_2 \qquad (3)$$

$$H + H^+ + e^- \text{ -----> } H_2 \qquad (4)$$

During corrosion the anodic and cathodic reactions are in balance, this is one of the reasons why corrosion current can not be measured directly. The center of attention of the corrosion process is often the anodic reaction because of the metal loss. However, the cathodic reaction can be slow or fast implying a control of the process and the metal loss. Consequently, any component of the corrosion system that influences reactions (3) and (4) will influence the cathodic reaction and hence the metal loss. These would involve compounds like phosphate that is included in most growth media in concentrations between 5 and 100 mM, and in the microorganisms themselves. The mineral solution used during this research contained 0.2 mM of phosphate. Results of the batch bioreactor shown in Figure 4-11 evidence the presence of a phosphorous compound in the form of a crystalline black

scale. These results agree with the above discussion and the findings of Iverson (1984) who suggested that a highly corrosive metabolic product during the microbial corrosion of steel by sulfate reducing bacteria was responsible for the blackening of the solution under a hydrogen atmosphere. X-ray powder pattern analysis of precipitated corrosion products indicated the presence of iron phosphide, he also indicated that the black film on the coupon was due to FeS. Results of the batch bioreactor also indicated the presence of a black precipitate suspended in the solution and attached to the metal caused by the activity of D. desulfuricans in the mineral solution. Figure 4-8 shows the corresponding EDXA spectrum that exhibits the presence of FeS. These results were very similar to the ones of pit coatings of FeS on mild steel in media containing sulfide ions found by Otero and Achucarro (1993). The results of this research and other findings elsewhere suggest that iron phosphate complexes and iron sulfide occur both in the bulk of the fluid and on the surface of mild steel.

The observation of cracks on SA106 steel coupons during experiments with electrochemical cells suggests the occurrence of hydrogen embrittlement. Figure 4-23 shows SEM (a) and b)) and EDXA (c) and d)) photomicrographs of SA106 steel coupons affected by longitudinal cracks along the surface. Steel coupons were used during the runs of the electrochemical cells

and developed cracks after more than four consecutive during the experiments.

The above results and the fact that hydrogen evolution from the steel proceeds via formation of atomic hydrogen, and that hydrogen may be absorbed on the steel surface, suggest that the cracks were the result of hydrogen embrittlement. Booth and Tiller (1968) reported that they were not able to account an apparent removal of hydrogen from mild steel and attributed this effect to a cathodic hydrogen evolution into the solution. This observation might have been associated with hydrogen embrittlement but they concluded that it was difficult for them to imagine another cathodic reaction available. Figures 4-24a and 4-24b are SEM photomicrographs of a characteristic crack at low and at high magnifications respectively. Figures 4-24c and 4-24d indicate EDXA photomicrographs showing the elemental distribution of corrosion products inside the crack and on the surface of the metal away from the crack.

The addition of fumarate to some of the electrochemical experiments accidentally elucidated the effects of a terminal electron acceptor on the hydrogen uptake activity of bacteria. Because of the difficulty of measuring the relatively small amounts of cathodic hydrogen produced by SA106 steel, fumarate was initially used as an alternative way to indirectly account for the amount of hydrogen uptake by the bacteria. It indirectly measures hydrogen uptake by means of

succinate formation during hydrogen uptake. Succinate is a more stable compound than fumarate (Anaerobe Laboratory Manual, 1975) and is easily measured in an ion chromatograph. It can also be measured in a gas chromatograph but requires a methylation procedure. Chromatograms show a very well defined peak for succinate after approximately 15 min of the injection of the sample. Fumarate has been tested successfully with E. coli with results showing 100% conversion to succinate during hydrogen uptake (Lee et al., 1985).

Experiments using methyl viologen (paraquat) or its analog benzyl viologen have demonstrated that those artificial electron acceptors may preferentially accept electrons from the atomic hydrogen first generated instead of electron from the hydrogen gas. This was demonstrated by adding methyl viologen to flasks that had a constant rate of hydrogen evolution. The results showed that as soon as the methyl viologen was added, the hydrogen evolution temporarily ceased until all methyl viologen was reduced. Thereafter, the hydrogen evolution approached the hydrogen evolution rate of the control. This effect was directly proportional to the concentration of methyl viologen used (Bryant and Laishley, 1989).

Results of this investigation presented in Figures 4-6 and Figure 4-8 have suggested that potential-time curves and hydrogen uptake characteristics are independent of the type and amount of five different hydrogen oxidizing bacteria.

However, when fumarate was added to some of the experiments with E. coli, it was observed that bacterial activity

Figure 4-23. Hydrogen embrittlement of SA106 steel in mineral solution electrolyte. a) SEM micrograph of crack at a low magnification

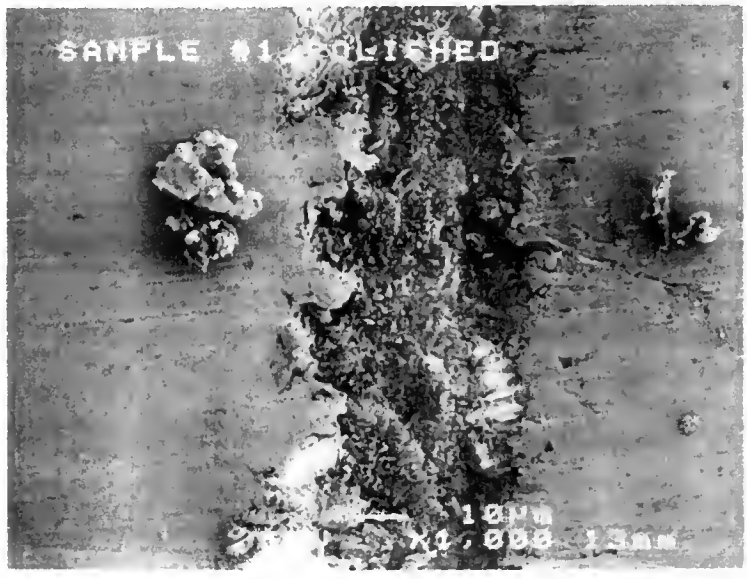


Figure 4-23. b) SEM micrograph of crack at a high magnification.

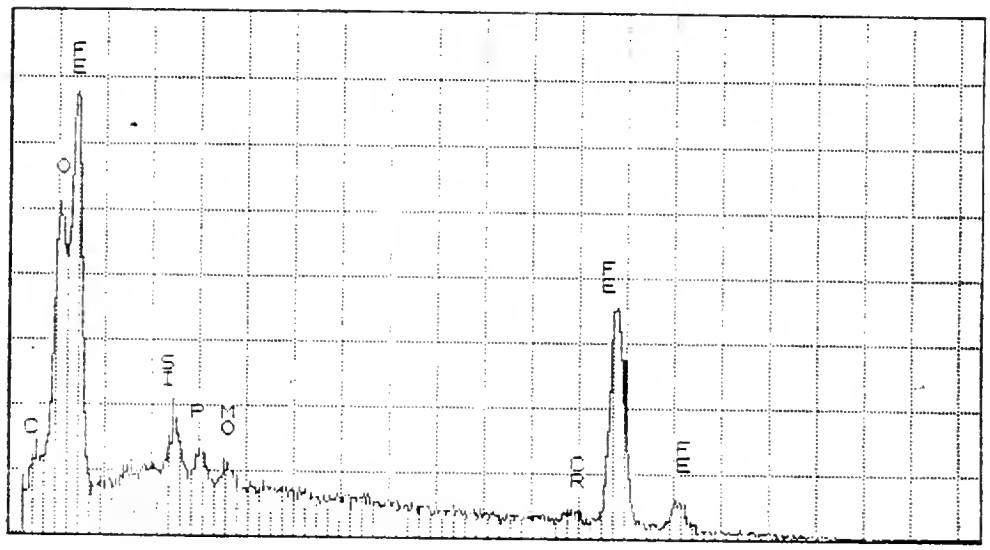


Figure 4-23. c) EDXA micrograph of corrosion products inside the crack.

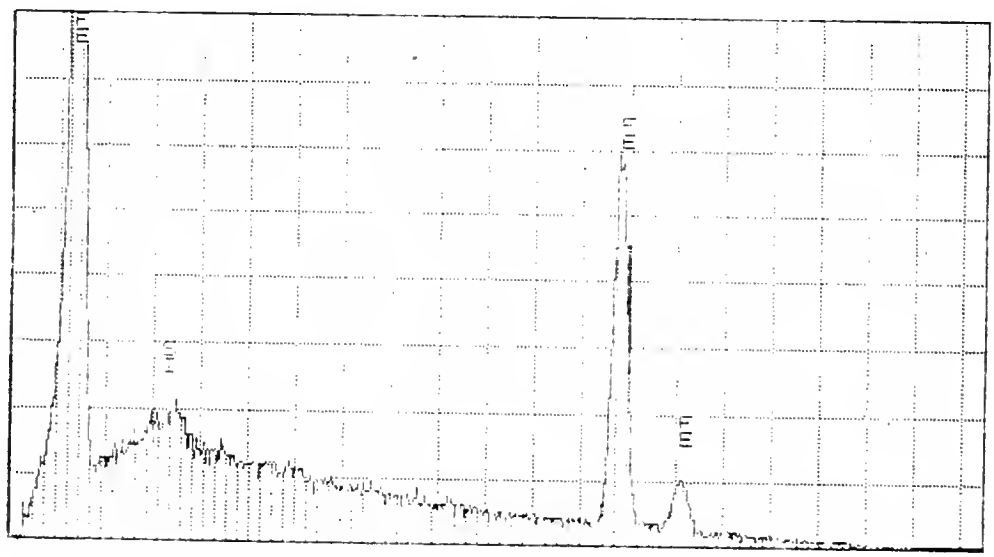


Figure 4-23. d) EDXA micrograph of uncracked control surface.

increased with corresponding increase in the differential corrosion current (DCC) and the concentration of dissolved iron. This fact suggested that in the absence of fumarate the hydrogen uptake rate is independent of the hydrogenase activity of the bacteria. This finding is supported by results of other researchers in the area (Booth and Tiller, 1968).

The hydrogen-fumarate medium has been recommended in the literature to be a defined medium used to test the ability of some bacteria to grow under anaerobic conditions utilizing hydrogen as electron source and fumarate as terminal electron acceptor (Lee et al., 1985). Its transformation is given in the following equation:

$$COOHCHCHCOO^- + 2\ H^+ + 2\ e^- \text{-----> } COOHCH_2CH_2COO^- \qquad (5)$$

Fumarate Succinate

Data reported from several investigators has shown that the hydrogenase enzyme influences the anodic reaction of the corrosion process by oxidizing the hydrogen produced. However, there is still much to be learned about hydrogenase at the physiological and biochemical levels. Hydrogenase is typically associated with the oxidation-reduction of various electron carriers. Positive evidence of cathodic depolarization by hydrogenase resulted from an experiment by Iverson (1966) in which a AISI1010 steel coupon in contact with masses of hydrogenase positive sulfate reducing bacteria on a reduced buffer agar surface containing benzyl viologen in

place of sulphate and electrically connected to another coupon isolated from bacterial cells through a micro ammeter. After 17 hours under a nitrogen atmosphere, the benzyl viologen under the coupon with the cells was reduced from colorless to purple color and iron dissolved under coupon isolated from bacteria cells suggested a catalytic effect on the activity of bacteria. In a similar manner, results of this research suggest that the reduction of fumarate influenced the activity of bacteria during their utilization of cathodic hydrogen because the fumarate as a terminal electron acceptor must have interacted with the bacterial hydrogenase system in order to increase their hydrogen uptake. Sadana and Morey (1961) in experiments with hydrogenase from sulfate reducing bacteria and methyl viologen proposed that the reduced form of the hydrogenase enzyme is more stable than its oxidized form.

Based on the fact that the chemical transformation of a thermodynamically stable compound to a less stable form involves a slower rate of reaction, and the fact that the rate of reaction of hydrogenase is faster in the presence of fumarase than in its absence; the presence of fumarate causes the enzyme to oxidize faster and hence uptake more hydrogen. Consequently, this may explain the observed increased bacterial activity in the presence of the terminal electron acceptor.

Hydrogenases are enzymes which are able to reversible catalyze the reactions involving hydrogen:

$$H_2 \text{ <===> } 2\ H \text{ <===> } 2\ H^+ + 2\ e^- \qquad (6)$$

The metabolism of hydrogen oxidizing bacteria is regulated by a combination of reversible hydrogenases located in the periplasm and cytoplasm of the organisms. It was after 1986 that three different hydrogenases were recognized for the sulfate reducing bacteria. A highly active Fe-containing periplasmic hydrogenase, a Fe-Ni-Se-containing periplasmic hydrogenase and a Fe-Ni-containing cytoplasmic hydrogenase. The activity of hydrogenase is affected by the concentration of Fe in the media (Czechowsky et al., 1990). The Fe-containing hydrogenases are believed to be restricted in distribution to strict anaerobic bacteria where information is limited or lacking (Adams, 1990). Recent research in the area indicates that different hydrogenases have different capabilities for hydrogen evolution and utilization and similar hydrogenases have different capabilities depending on their location in the cell. For example, the dimeric Ni-containing hydrogenase is periplasmic for Desulfovibrio gigas and has an specific activity of 1400 for hydrogen utilization and 400 for hydrogen evolution. In the case of E. coli this hydrogenase type is transmembranic and has an specific activity of 38 for hydrogen utilization versus 76 for hydrogen evolution (Przybyla et al., 1992). Those findings throw new light on the understanding of the complex microbial corrosion phenomena.

In addition to the above discussion most researcher fail to consider the myriad of different factors that can affect cathodic depolarization. For instance when surface scaling adheres to metal surfaces depolarization of the cathode is not as easily reversible as hydrogen depolarization. The study of microbial utilization of cathodic hydrogen would be much easier avoiding the formation of such products. This research, besides providing conditions to avoid the formation of any film and/or electrochemical external effect, also focused on the onset of the corrosion process. The beginning of the corrosion process may predict how the complex multiple dynamic microbial corrosion process will influence the later stages. In practice, experimental determinations of corrosion currents always induce electrochemical changes in the corrosion system and this is undesirable. On top of that, it is important to consider that steel corrodes spontaneously in aqueous environments such as the mineral solution; and the presence of mild scale, irregularities in the crystalline structure, and any other variation in the physical chemical nature of the surface such as grain boundaries and inclusions, will result in the metal structure becoming micro anodic and cathodic areas that may result in some forms of localized corrosion.

New Proposed Microbial Corrosion Mechanism

Overall results of this research afford evidence of cathodic hydrogen utilization by hydrogen oxidizing bacteria during anaerobic corrosion of carbon steel. They provide evidence of several findings that allows the proposition of a comprehensible new microbial corrosion model. This model focus on the on-set of the microbial corrosion process and builds on the theory of cathodic depolarization described in Chapter 2. Figure 4-24 depicts the proposed new microbial corrosion mechanism. The most important findings of this investigation are enclosed in the model and include the evidence of: 1) hydrogen production by metals in the mineral electrolyte solution, Table 4-3, 2) positive influence of bacterial activity in the corrosion of metals indicated in the potential-time curves, dissolved iron profiles and differential corrosion current measurements, see Figures 4-15 through 4-20, 3) SEM and EDXA spectra of corrosion products such as FeS and acetate, resulted from the activity of bacteria, Figures 4-1 through 4-12 and Table 4-1, 4) hydrogen utilization by bacteria, Tables 4-6 and 4-7, 5) effect of a terminal electron acceptor in the activity of bacteria indicated in the results of experiments with the dual cell and with the triad cell, Table 4-9 and Table 4-10, 6) hydrogen embrittlement observed on SA106 steel coupons during experiments with the electrochemical cells, Figure 2-23, and

7) approach to measure the biological component of corrosion suggested by the triad cell results.

The new anaerobic corrosion mechanism suggests that metallic iron oxidizes and dissolves in the acidic mineral

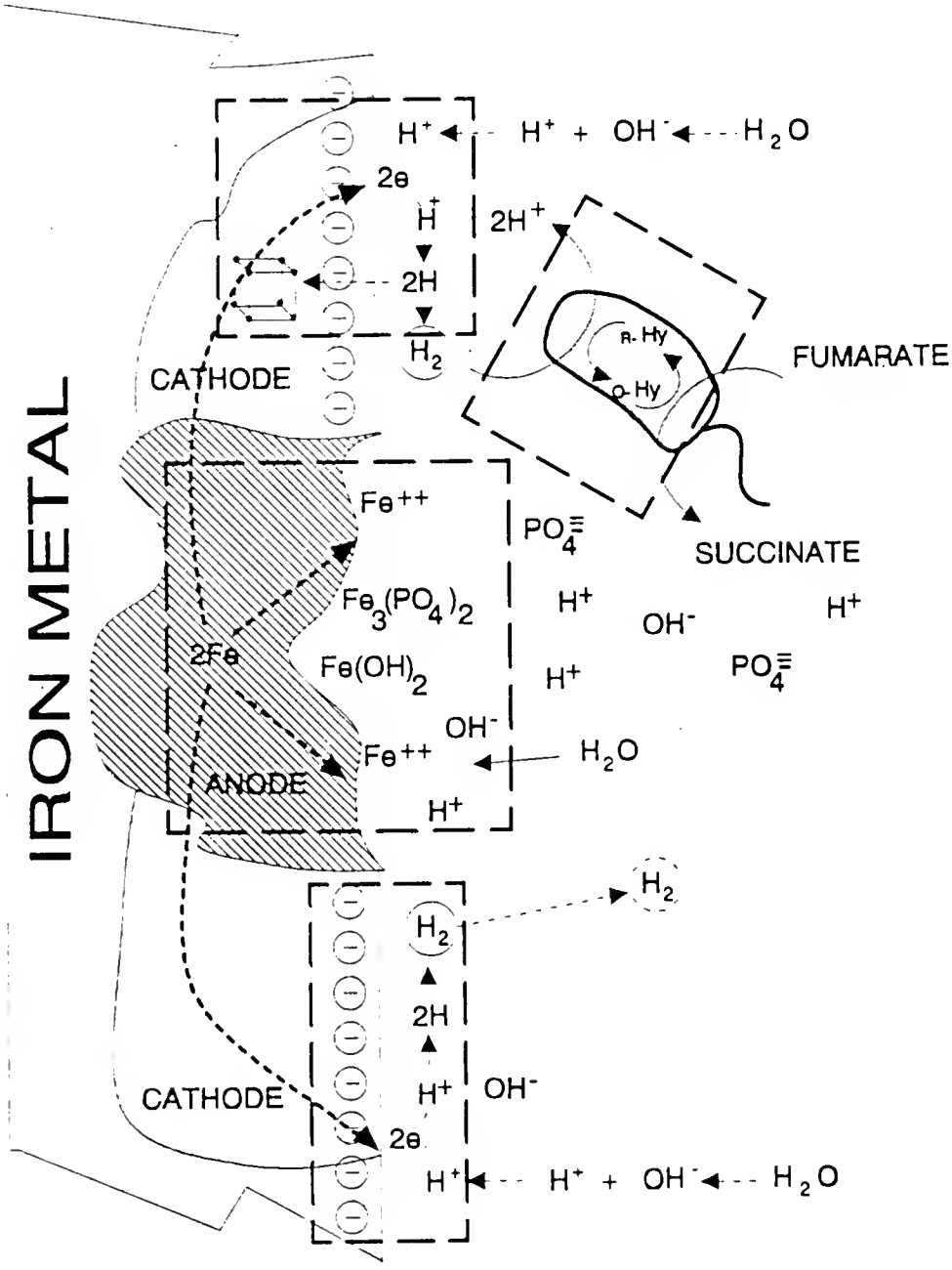


Figure 4-24. Proposed anaerobic mechanism for the on-set of microbial corrosion of steel by hydrogen oxidizing bacteria.

electrolyte solution. During the process electrons from the metal are released to cathodic sites. Hydrogen ions resulting from water dissociation migrate to the negatively charged metallic surfaces and become reduced with the excess electrons accumulated at cathodic sites. Atomic hydrogen being produced at the surface is either absorbed into the metallic crystalline structure or recombines itself with additional atomic hydrogen and produces molecular hydrogen. Absorbed hydrogen may cause hydrogen embrittlement and molecular hydrogen may either cause cathodic polarization or diffuse into the bulk of the fluid and head space. Molecular hydrogen is then taken up by the hydrogenase system of the hydrogen oxidizing bacteria. The presence of a terminal electron acceptor increases the hydrogenase activity and hence increases the hydrogen uptake by bacteria. As a result, more cathodic hydrogen is produced and consequently more metallic iron is oxidized to maintain the overall electroneutrality.

Capabilities of the Triad Cell

The triad cell was designed with the objective of simulating the on-set to study microbial utilization of cathodic hydrogen from mild steel and for the determination of most of the parameters suggested in the proposed microbial corrosion model described in Figure 4-24. It is the conviction of the author that with the proper determination of

those parameters and some auxiliary analysis such as SEM, metallography, and others; it may be feasible to define the microbial corrosion mechanism of a particular situation to be studied in the triad cell, and the correlation of the measured parameters elucidate quantitatively the biological component of corrosion.

The triad cell could be compared to a short circuited battery between flasks I and III where the differential corrosion current (DCC) measured is the result of the potential difference caused by the activity of the bacteria influencing the corrosion of the metal coupon in flask I.

Potentials are practical indications of the corrosion state of a metal in an electrolyte and depends on the electrode reactions. It is a simple measurement that requires a millivoltmeter, with a high impedance to prevent current drain during measurement, and a reference electrode capable of providing a stable reproducible potential. It is impossible to measure absolute potential, for this reason an arbitrary reference electrode standard is chosen such as the hydrogen electrode that is assigned a potential of 0.00 V when hydrogen activity is 1.0 M and its partial pressure is one atmosphere. The free corrosion potential is measured by placing the metal coupon and the reference electrode in the electrolyte and reading the meter in volts. In practice, the free corrosion potential, is the voltage developed between the corroding metal and the reference electrode in contact with the

corrosive environment. While one reaction is dominant in the boundary layer of the reference electrode, more electrode reactions may be proceeding in the boundary layer of the corroding metal coupon. As a result, the measured voltage is the sum of two voltages developed in the double layers of both electrodes. It is really a mixed potential in the electrochemical sense. The free corrosion potential is also a dependent variable in corrosion and electrochemical measurements and it is connected to corrosion rate by simple functions under fixed specific conditions. If a consistent free corrosion potential difference between a metal coupon exposed and unexposed to bacteria could be determined, this might be correlated with the biological component of corrosion. In the case of the single and dual electrochemical cells, the rationale was to quantify a free corrosion potential difference which can be attributed to the influence of bacteria during the corrosion of a metal, and correlate that to corrosion rates (corrosion current density) via classical electrochemical corrosion theory such as the Butler-Volmer and Nernst relationships. In this manner, a theoretical corrosion rate might be calculated, avoiding the classical electrochemical procedures that require potentiostatic and/or potentiodynamic techniques that usually introduce external effects to the microbial corrosion system.

Corrosion rate or corrosion current is probably the most informative and most desirable parameter to know about the

deterioration of a metal. However, the literature indicate that it is not possible as yet to measure such a parameter directly, but experimentally as a function of potentials. Some experimental techniques include mass loss measurements, linear polarization, and impedance spectroscopy. Some new technologies such as the quartz microbalance could give mass loss information in real time. The triad cell offers to measure a differential corrosion current (DCC) directly, and this may be able to be correlated to the total corrosion rate of the metal and to the biological component of corrosion. Total corrosion is the integrated function of corrosion rate over the time period considered for the experiment. The units of corrosion rate are equivalent to current density by Faraday's law. Total corrosion in a circumstance of microbial corrosion includes the corrosion of the metal in the absence of bacteria and the corrosion influenced by microorganisms.

The triad cell allows for the measurement and data acquisition of the following parameters: pH, potential, oxidation-reduction potential, temperature, DCC, and head space pressure. It also allows the sampling of the head space gas, liquid media and metal coupon for supplemental analysis such as gas composition, volatile fatty acids, dissolved iron and other ions, absorbance, metallography, microscopy, and analysis of corrosion products. A list of recommended implementations to improve the triad cell are included in the suggested research section.

The experimental results shown in Figure 4-22 may be analyzed by comparing it with a Daniel Cell in which the Zn electrode is replaced with a Mg electrode and the solution of the salts for the mineral solution electrolyte. The electrical current produced by the corrosion reaction may be compared to the differential corrosion current measured in the triad cell. "The Daniel Cell was a very early form of battery which consisted of Cu and Zn immersed in a solution of their salts" (Trethewey and Chamberlain, 1988).

CHAPTER 5
SUMMARY AND CONCLUSIONS

Summary

Microbial corrosion is a pervasive and widely recognized problem of massive proportions in many industrial processes worldwide. In particular anaerobic corrosion of steel is of great economic importance. Much remains to be learned about basic mechanisms and measurement of the influence of those organisms in the corrosion process if we are to mitigate and control its catastrophic effects. The techniques available to control the organisms and/or protect the metal are in many cases inadequate. Microbial corrosion requires the understanding of several scientific disciplines in order to provide insight into its study. There is a need for experimental systems which provide quantitative measurements of the influence of bacteria in the corrosion process. Non-disruptive electrochemical techniques combined with physical-chemical analysis, in a new batch bioreactor, appears capable of measuring the biological component of anaerobic corrosion from the very early stages of the process. The technique involves the use of a "triad flask" electrochemical cell, Figure 3-6, that indirectly measures microbial utilization of cathodic hydrogen using a terminal electron acceptor and relates it to free corrosion potentials, differential

corrosion currents, and corrosion rates calculated from dissolved iron analysis.

The overall objective of this investigation was to develop a method to study microbial corrosion from its very early stages by measuring the microbial utilization of cathodic hydrogen. It is known that certain microorganisms consume hydrogen in their metabolic processes. Since hydrogen embrittlement of metallic structures is a corrosion consequence of inmense economic proportions, the present study was designed to explore the feasibility of integrating hydrogen embrittlement (by consuming the hydrogen) through microbiological processes. This secondary task focused on attempting to quantify the biological component of corrosion.

Two new flowthrough and batch bioreactor systems were employed. The flowthrough bioreactor is able to reproduce microbial corrosion of carbon steel in the presence of a known bacteria triculture and glucose-lactate yeast extract media. During the first week, scanning electron microscopy revealed that the bacteria triculture composed of Enterobacter aerogenes, Clostridium acetobutylicum and Desulfovibrio desulfuricans produced a thick, and dark biofilm that consisted of a double layer polymeric structure and a random distribution of pitting on the metal surface. Electron dispersion X-ray analysis (EDXA) analysis provided elemental analysis of the corrosion products resulting from the interaction of metabolites produced by bacteria on the surface

of the steel. Volatile fatty acids analysis indicated the presence of acetate, a metabolite, reported to be responsible for the corrosion of steel even in very low concentrations.

The batch bioreactor produced microbial corrosion within 24 hours. The various microbial species present are similar to those encountered in natural environments. SEM analysis confirmed that the bacteria triculture caused pitting corrosion, formed a biofilm on the metal surface and produced a black precipitate of FeS on the bulk of the fluid, and on the metal surface. Among other advantages, it allowed for visual inspection, it was less prone to contamination than the flowthrough bioreactor, and it offered a better control of the experiments. Experiments with the batch system to study the carbon and energy sources for the bacteria triculture show that head space gas composition affects the bacteria/metal/fluid system. For example, bacteria metabolize the head space gases nitrogen, hydrogen and methane in their metabolic processes. Results show that D. desulfuricans and phosphate were responsible for the black precipitate suspended the liquid and deposited on the metal surface.

An experimental system based on the batch bioreactor is intended to simulate conditions inside of gas transmission pipelines. Electrochemical measurement capabilities was provided to attempt to quantify the biological component of corrosion. The experimental system was instrumented to a five channel data acquisition system which recorded on-line data

including pH, free corrosion potential, oxidation-reduction potential, head space gas pressure, and diferential corrosion current in the presence and absence of bacteria under controlled environmental conditions. Conventional physical-chemical analyses were also performed periodically using "grab" sample.

The experimental system results indicate that the response of the potential-time curves to the presence of bacteria were more reactive, but were not significant enough to determine the influence of bacteria in the corrosion of steel. Among the reasons were the small magnitudes of the potential differences when compared to control runs without bacteria, the similarities of results obtained using five different bacteria, and different bacterial concentrations at different stages of growth. Results of dissolved iron concentration profiles were consistent with the potential-time curves, indicating a higher corrosion for experiments with bacteria.

Final experiments using the triad cell included measurements of a differential corrosion current. These experiments produced comparable results to the potential-time curves and measurable DCCs were observed when the bacteria were present. The results of this experiment were also inadequate to calculate the biological component of corrosion.

During the electrochemical experiments, two important findings were observed: the detection of longitudinal cracks

along the surface of the steel coupon due to hydrogen embrittlement, and the increased activity of bacteria during the addition of a terminal electron acceptor that suggest a catalytic effect on the hydrogenase activity of bacteria during the utilization of cathodic hydrogen. These findings along with other ideas were incorporated in a new proposed MC model. Significant increases in the calculated corrosion values from dissolved iron analysis and the DCC, were observed during the addition of the terminal electron acceptor.

Despite the difficulties in quantifying the biological component of corrosion, this research brings about deeper insight into the overall understanding of the phenomena involved in microbial utilization of cathodic hydrogen; and concepts developed herein provide another stepping stone on the path towards a full understanding of microbial corrosion.

Conclusions

An attempt has been made to determine the influence of bacteria during the anaerobic corrosion of carbon steel at its onset, using a new triad electrochemical cell. Preliminary experiments using two new bench scale flowthrough and batch bioreactors reproduced microbial corrosion. The batch system resulted in superior operations and was redesigned to include electrochemical measurements. this then evolved into the triad electrochemical cell. Despite the small and

inconsistent potential and deferential corrosion current differences of steel coupons exposed and unexposed to bacteria, which discourage the quantification of the biological component of corrosion; the overall results afford strong evidence of the influence of bacteria on the cathodic depolarization of carbon steel. Furthermore, the observation of hydrogen embrittlement in carbon steel coupons in the electrochemical cells, and the closed examination of the catalytic effect of a terminal electron acceptor on the activity of bacteria during utilization of cathodic hydrogen at the onset of corrosion suggested a new microbial corrosion mechanism. In general, this research brings about a new approach to the investigation of microbial corrosion and the concepts developed herein provide better insight to the overall understanding of this pervasive and costly problem.

Some detailed conclusions follow:

1) The flowthrough bioreactor reproduced anaerobic corrosion of carbon steel. Of particular interest was the formation of the biofilm that involved the formation of a double layered structure.

2) The batch bioreactor was able to reproduce microbial corrosion within 24 hours and resulted in a superior system to study bacterial utilization of cathodic hydrogen at its onset, of particular interest were: a) the head space pressure drop measurements that suggested microbial metabolism of nitrogen, hydrogen and methane, b) a black FeS precipitate formed on the

surface of the metal and in the bulk of the liquid as a result of sulphate reduction by Desulfovibrio desulfuricans and the corresponding increase of phosphate contained in the media.

3) Potential-time curves results afford strong evidence regarding the influence of bacteria in the deterioration of carbon steel.

4) The differential corrosion current measurements corroborated previous reults of potential-time curves.

5) SA106 steel is susceptible to hydrogen embrittlement.

6) The use of fumarate as terminal electron acceptor in the electrochemical cell suggested an increased activity of the bacteria. Differential corrosion current and dissolved iron concentrations were at least doubled immediately after its addition.

7) The concepts developed during this investigation were included in a new anaerobic mechanism that descrives the onset of microbial corrosion.

Suggested Future Research

The author suggest continuation of the research effort in the following specific areas.

1) Bacteria triculture metabolism of the head-space gases nitrogen, hydrogen and methane in rich and poor nutrient media at basic, neutral, and acidic pH values.

2)The effect of terminal electron acceptors on potential-time curves for SA106 steel in mineral solutions in the presence of different cell concentrations and age of E. coli and their hydrogenase mutants, including continuous culture.

3) Effect of terminal electron acceptors on the activity of hydrogenase from dead cells of hydrogenase positive bacteria, during the corrosion of SA106 steel in mineral solution.

4) Test of the triad cell provided with the following instrumentation: a) 15 channel data acquisition system able to measure pH, pressure, temperature, oxidation-reduction potential, potential, and current; b) a rigid triad flask glass cell; c) an IBM compatible computer with a hard drive; d) a mechanical device that allows the raising and lowering of the metal coupons in the electrolyte; e) precise liquid and gas sampling ports; f) leak proof system to assure anaerobic conditions throughout the experiments; and g) a larger water bath with temperature control.

5) Effect of phosphate in the microbial corrosion of SA106

steel in the presence of sulphate reducing bacteria and emphasis on the formation of the black precipitate.

6) Effect of metallic microstructure on the production of cathodic hydrogen.

7) Effect of grain boundaries on bacterial adhesion.

8) Hydrogen embrittlement of SA106 steel in mineral solution.

9) Effects of head space changes in the triad cell, using similar and dissimilar metals.

10) Correlation of differential corrosion current, hydrogen uptake, succinate formation, total corrosion and potential changes in the triad cell using SA106 steel and a hydrogenase positive bacteria.

11) Item 10 using Mg.

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BIOGRAPHICAL SKETCH

Jose Rafael Sifontes Garcia was born February 16, 1949, in Santa Rosa, Anzoategui, Venezuela, to Mr. and Mrs. Pedro Rafael Sifontes as the first born of two sons. Since early in school he has shown to be an exceptionally gifted person. From primary school to high school, he demonstrated his scholastic excellence by achieving the #1 rank in all his classes, receiving also the highest high school award during his graduation in 1967 from Pedro Emilio Coll High School, Caracas, Venezuela, for having attained the highest academic performance. The same year he was awarded the Mobil Oil Co. National Fellowship to study abroad, but he could not accept it because of family sentimental values. He started his university career at the Universidad Central de Venezuela, where he advanced for two years until the 1969 political turmoil that led the government to close down the institution. Mobil Oil Co. offered the fellowship again, and he transferred to the University of Kansas (KU), as an advanced student, to continue his studies in chemical engineering. In four years at KU he learned English and obtained bachelor's degrees in chemical engineering and in chemistry, and a Master of Science in Petroleum Engineering with a minor in business administration. During his stay at KU, he also was on the

dean's list, participated in the leadership of the AICHE, and was recognized by the Kansas Geological Survey. Upon completion of his education he started working for Mobil Oil Co. El Palito refinery in Venezuela in 1974. Months later he also started teaching at the University of Carabobo during the evenings. In 1978 he was offered a management position with the Dowell Schlumberger Industrial Services Division. He advanced positions rapidly in those organizations: at Mobil he advanced from process to planning engineer; at Dowell from sales engineer to national sales manager; and at the University of Carabobo from part-time instructor to department chairman and full professor. During all this time, his involvement with industry, community, and concern for the environment awoke his desire to pursue a multidisciplinary education to help understand and solve the challenging problems of our times.

During his high school years, Jose started competing in athletics, baseball and soccer. He continue playing soccer after high school and joined a professional soccer team in Caracas. He also played the guitar for the high school group Estudiantina del Colegio San Antonio. He was a member of the Christian group Juventud Catolica, which interacted with the community in sports and musical events during Christmas. While in the USA, he became involved in sports at KU, where he learned and represented the university in judo, fencing, and ping pong. He also practiced tennis, volley ball, and golf

for fun. He work for the KU police department under the supervision of Mr. Ian Davis and joined student organizations at KU and held leadership positions in AICHE.

While working at the Mobil Oil Co. refinery, he played soccer and tennis, representing also the Universidad de Carabobo. While teaching, he became aware of environmental issues. Among his contributions were development of the course Environmental Conservation for Chemical Engineers and help with the initiation of a national environmental movement, in accord with professors in other Venezuelan universities. This movement produced the Environmental Federation, which later led to the approval of the actual environmental laws for air and water in Venezuela. It was after visiting the University of Florida in 1981 that he decided to continue his education. Since his arrival at the University of Florida, he has polished and matured his knowledge and experience in life, proving his outstanding scholastic record and his admirable example of service and leadership in and out of the university. He has graduated twice from the Environmental Engineering Department, receiving M.E. and B.S. degrees with honors, and from the Materials Science and Engineering Department, receiving an M.E. degree. In June 1989, he started pursuing the Doctor of Philosophy degree in the Agricultural Engineering Department. He concentrated his course work and research in the area of microbial corrosion. His project work included the development and implementation

of a triad flask electrochemical cell to measure the biological effect in the corrosion process and a new microbial corrosion model. His overall graduate GPA is 3.6.

Among his contributions to the University of Florida, he has served in the Mentor Program as peer mentor, on the Silver and Gold Committee representing Golden Key National Honor Society, in the SECME Olympiad as a UF representative, in the Environmental Graduate Student Association as social director and treasurer, with the Affirmative Action Round-table as student representative, in the Hispanic Assembly as ambassador, and in the Hispanic Engineering Society as Engineering Fair chairman. Among his most recent awards are the 1992 National Bill Nuanes scholarship from the Society of Hispanic Professional Engineers; 1992 and 1993 UF Presidential Recognition; 1992 Minority Student Recognition; 1991 and 1992 Florida College Student of the Year recognition; 1992 and 1993 Who's Who Among Students in American Universities and Colleges; 1991, 1992, and 1993 Highest Honors awards from the Southeastern Bible Institute. Jose is also a member of the following campus honorary organizations: Omicron Delta Kappa, the National Leadership Honor Society; Alpha Sigma Mu, the International Metallurgical Honor Society; Alpha Epsilon, the Honor Society of Agricultural Engineering; Golden Key National Honor Society; Gamma Sigma Delta, the Honor Society of Agriculture; and Tau Beta Pi, the National Engineering Honor Society.

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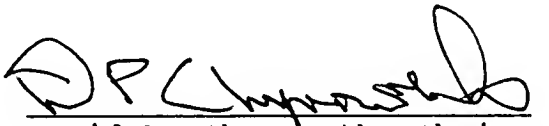

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Professor of Agricultural
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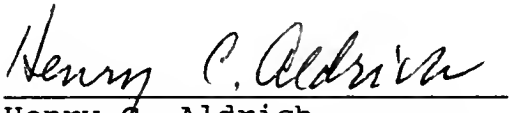

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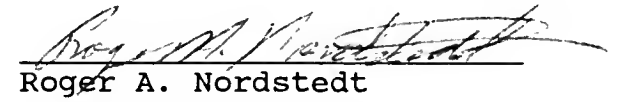

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This dissertation was submitted to the Graduate Faculty of the College of Engineering and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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